AN INVESTIGATION INTO THE USE OF SYNTHETIC ZEOLITES FOR *IN SITU* LAND RECLAMATION.

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ABSTRACT

This thesis describes an experimental study to determine the feasibility of using zeolite addition for the *in situ* treatment of soils contaminated with heavy metals. The aim of the present work was to examine the effectiveness of three synthetic zeolites to reduce plant available metal pools in contaminated soils. Three contaminated soils were studied, which are representative of typical contamination sites in the UK: Prescot, site of a copper refinery, Trelogan, an old lead/zinc mine spoil, and Gateacre, a sewage sludge treated field.

The action of zeolites to reduce available metal concentrations in soils is due to their ion-exchange properties. To investigate the decrease of metal bioavailability by zeolites, laboratory and greenhouse trials were performed to clarify the mechanism of heavy metal fixation by synthetic zeolites and to quantify the effect of different zeolites for land remediation. For this approach, it was necessary to measure the metal concentration in the soil and the soil solution in zeolite-amended soils and to determine the zeolite specific isotherms of all the metals studied.

Cation exchange studies involved exchanging the sodium form of the zeolites with different metals in solution, in order to determine the zeolite affinity for the metals copper, cadmium, zinc and lead. The resulting isotherms demonstrated that all three zeolites showed a preference for the heavy metal ions over sodium ions.

The changes in metal speciation in zeolite-treated contaminated soils were evaluated using sequential extraction procedures. After incubation with synthetic zeolites, metals extracted with ammonium acetate were significantly decreased (31.4 % - 72.4 %) in amended soils compared to the controls. This decrease in heavy metal availability is extremely significant. The exchangeable metal fraction is the most available for uptake by plants.Long-term soil solution experiments with zeolite-amended soils showed that the metal concentrations in the aqueous leachate were significantly reduced than in the leachate from the same substrates without zeolite addition.

Greenhouse pot trials were carried out with sunflower (*Helianthus annuus*), maize (*Zea mays*), willows (*Salix viminalis*) and ryegrass (*Lolium perenne*) plants grown in zeolite amended contaminated soils. There were significant improvements in visual appearance and growth of plants from the zeolite-treated soils compared to the controls. In addition, metal content of plant tissues was reduced when compared to the controls. Optimum zeolite concentrations were noted for each zeolite. Zeolite P and 4A were

more effective at reducing the phytotoxicity at 0.5% and 1%, whilst zeolite Y had to be added at 5% to achieve a similar effect.

In order to link the laboratory test results and soil data to a pilot field scale, in which the actual soil and environmental conditions are required to give a complete evaluation of the proposal technique when applied to a given hazardous waste site, a field trial was initiated, at a copper contaminated site at B.I.C.C., Prescot to examine the effectiveness of zeolite amendments under field conditions. Zeolites P and 4A applied at 1% level proved to be an effective treatment for the remediation of the contaminated site, as indicated by improved plant growth and low metal concentrations in the water soluble fraction of the soil.

The results show that zeolite addition, particularly zeolites P and 4A, provide an effective method for decreasing plant heavy metal bioavailability in polluted soils, under glasshouse and field conditions.

CHAPTER 1.
INTRODUCTION

1.1. Contaminated land, particularly Merseyside and N.West.

Contaminated land is defined as land that contains substances that, when present in sufficient quantities or concentrations, are likely to cause harm directly or indirectly to humans and the environment (NATO Committee on the Challenges of Modern Society, 1993). Neither the total area of contaminated land in the UK, nor the number of sites, can be quantified precisely at this moment, although an independent estimate suggested that the area is in the range of 50,000 to 250,000 ha (Timothy, 1992) (Martin and Bardos, 1994).

Abandoned mine workings and other industrial wastes associated with the exploitation of non-ferrous metal ores are ubiquitous in the United Kingdom. Derelict buildings and mineral spoil heaps are typical features of the landscape in areas of Central/North Wales, south-west England and the Central/North Pennines. The production of lead, zinc, copper or tin ores was centred on these areas during the period 1700-1890 (Lewis, 1967). In Wales alone there are some 2000 ha of metal-contaminated derelict land comprising mainly waste products discarded during the separation of valuable metals (chiefly lead and zinc) from the associated gangue minerals in crude ore matrices.

Plant growth and development on metalliferous mine spoil is restricted by the high concentrations of phytotoxic metals and by the low levels of macronutrients in the substrates.

Natural revegetation is restricted by residual quantities of toxic metals in these substrates and by their inherent low fertility (Smith & Bradshaw, 1972). Many spoil tips are therefore unstable and redistribution of spoil by flooding of contaminated watercourses (Davies & Alloway, 1970) and by wind erosion (Smith, 1973) has caused extensive despoilation of agricultural land adjacent to derelict mine sites.

Many derelict mines are situated in areas of outstanding natural beauty (e.g. National Parks) which are subjected to considerable recreational pressures. The unattractive appearance of these derelict mines is accentuated by the absence of a vegetation cover and, in several cases, by uncontrolled disposal of other industrial, domestic or agricultural wastes. A permanent, economically viable solution is required which will both minimise the potential pollution hazards and enhance the low amenity value of these sites (Johnson *et al.*, 1977).

1.2. Problems of metal pollution

Soils may become polluted with high concentrations of heavy metals both naturally, as a result of proximity to mineral outcrops or ore bodies, or anthropogenically, arising from industrial activities. Metalliferous mining and processing, including the uncontrolled disposal of wastes, usually produces the most severe cases of heavy metal pollution. Other operations such as smelting, waste and effluent disposal have however dispersed metal contaminants very widely from their sources of generation, although at lower concentrations (Mitchell and Atkinson, 1991).

Compared with natural soils, metalliferous wastes exhibit physical and biological characteristics which may prevent the re-establishment of locally dominant plant species without prior remedial measures. The wastes are also a source of ground and surface water pollution, caused by the presence of heavy metals. Common problems associated with the waste include narrow particle size distribution, compaction, absence of beneficial macro- and micro-organisms, plus the lack of essential plant nutrients and organic matter. These characteristics can combine to inhibit germination, stunt plant growth, and cause premature death. Equally important is the presence of heavy metals in the form of chemically reactive minerals and related weathered products. Without vegetative cover, metal sulphides are susceptible to oxidation, producing sulphuric acid and water-soluble metal sulphates. Waters permeating the contaminated land become acidified and may dissolve the sulphates, sometimes dissolving several hundred parts per million of copper, zinc and other heavy metals. Theoretically the metals ions may be carried over large distances, however it is more likely that they will be reabsorbed onto the surface of clay particles or organic matter by ion-exchange or complexation, or reprecipitated as the groundwater pH increases. Despite this, because of the size and number of sources, contamination can cover wide areas and can still spread a considerable distance from its primary source. Additional dispersion may also occur by wind removal of unconsolidated nonvegetated areas, particularly from fine-grained waste or tailings (Alloway and Jackson, 1991).

1.3. Legislation

UK policy on contaminated land is not a new phenomenon. It has evolved over a 18 year period during which various measures (such as planning and building controls,

environmental protection and public health legislation) have been used to identify contaminated sites and provide for their safe and beneficial re-use.

In 1990 the Environmental Protection Act (EPA) was issued. Two sections of EPA are of relevant importance for land remediation: Section 143, concerning the duty of local authorities to compile registers of land which is, or has been, subject to contaminative use, and Section 61, regarding the duty of waste regulation authorities to inspect land which may cause pollution of the environment or harm human health (Draft Environmental Protection Act, 1992).

Also in 1990, the DoE established a comprehensive research programme on contaminated land. The research programme covers a wide spectrum of issues from methods of accurately identifying, characterising and assessing the risks associated with contaminated land, through the selection, design and implementation of remedial works, to quality assurance considerations and the legal and other implications associated with the sale and transfer of contaminated land. This research programme provides a welcome foundation supporting both the further development of UK contaminated land policy and the safe, effective and economic management of contaminated land in practice (Harris, 1993).

In recent years legislative criteria have developed worldwide on the basis of those provided by " Best Available Technology " (BAT). Sometimes, as in UK, this has been modified by economic and managerial factors to " Best Available Technique Not Entailing Excessive Cost " (BATNEEC). Integrated Pollution Control requires all wastes and emissions to be reduced to the practicable minimum by the use of BATNEEC (Barbour, 1994).

The EC Directive 76/464 for water pollution, which concerns the discharge of Dangerous Substances into the Aquatic Environment, specifies a number of broad families of chemical substances, dividing them into two Lists: List I, containing those considered most toxic (the 'black list'), and List II (the 'grey list'), containing those that are less dangerous (Table 1.1.). The stated aims of the Directive are to 'eliminate' pollution caused by List I substances, and to 'reduce' pollution caused by List II substances.

As a member of the European Community (EC) the UK Government is required to comply with EC legislation. In the UK, as elsewhere in Europe, all political parties are becoming 'greener', partly in response to public concern about specific topics such as deterioration of the ozone layer, acid rain, and the greenhouse effect, and partly due to an anti-industry view (Harrison, 1992).

Table 1.1. Black and Grey List Chemicals (adapted from Harrison, 1992).

List 1 (Black List)	List 2 (Grey List)
List 1 Substances	List 2 Substances
organohalogen compounds and substances which may form such compounds in the aquatic environment;organophosphorus compounds; organotin compounds; carcinogenic substances; mercury and its compounds; cadmium and its compounds; persistent mineral oils and hydrocarbons of petroleum origin; persistent synthetic substances which may float, remain in suspension or sink.	The following metalloids and metals and their compounds: zinc, copper, nickel, chromium, lead, selenium, arsenic, antimony, molybdenum, titanium, tin, barium, beryllium, boron, uranium, vanadium, cobalt, thallium, tellurium, silver. Biocides and their derivatives not appearing in List I; substances which have a deleterious effect on the taste and/or smell of the products for human consumption; toxic or persistent organic compounds of silicon; inorganic compounds of phosphorus and elemental phosphorus; non-persistent mineral oils and hydrocarbons of petroleum origin; cyanides, fluorides; substances which have an adverse effect on the oxygen balance, particularly: ammonia, nitrites.
Chemicals selected for control as List I Chemicals	Control for List II
mercury; cadmium; hexachlorocyclohexane; carbon tetrachloride; DDT; pentachlorophenol; "drins" (aldrin, dieldrin, endrin, isodrin); hexachlorobenzene; hexachlorobutadiene; chloroform.	using the EQO (Environmental Quality Objective) approach using quality standards set nationally.
Candidate Chemicals for List I	List II Chemicals for which UK National Standards have been set
chlorinated hydrocarbons; chlorophenols; chloroanilines and nitrobenzene's; polycyclic aromatic hydrocarbons; inorganic chemicals; solvents; pesticides.	lead; chromium; zinc; copper; nickel; arsenic; boron; iron; vanadium; tributyltin compounds; triphenyltin compounds; cyfluthrin; sulcofuron; flucofuron; permethrin.

Governmental directives are generally based on total metal contents permitted in soils. However, for plants growing on these soils and for other biota directly or indirectly feeding on these plants, total soil metal content only has relative significance since various other physical and chemical soil parameters (pH, cation exchange capacity, chemical form of the metals, organic matter content, etc.) determine the metal availability to the plant root system and the transfer of metals from the abiotic to the biotic compartment of an ecosystem. Soil metal content does not necessarily mirror the plant metal bioavailability (Juste and Tauzin, 1992). Government legislation takes into account only the total metal content, but it is the metal species which needs to be taken into account.

1.4. Methods for remediation

Past industrial activity and inappropriate management of waste has left a legacy of polluted sites which are now a major subject of concern for environmental protection in industrialised countries. As a result, political and administrative action has been taken and financial resources made available. Within the rehabilitation projects that are carried out, the treatment of polluted soils constitutes a key element that has required, and still requires, the development of new specific techniques (Table 1.2.).

There are several options available for the remediation of contaminated sites. The choice of option will depend on the nature of the contaminants, the type of soil, the characteristics of the site, its intended use, the relative costs of the appropriate options, and the regulations which apply in the country or region where the site is located. The remediation options can vary from the minimum of reducing the bioavailability of the contaminants, to the maximum of either complete clean-up of the soil, or its removal from the site.

Soils around metallurgical industries can be highly polluted by metals such as cadmium, zinc, lead, copper, etc., due to the large emissions resulting from past metallurgical production processes. Extremely high contamination, in combination with specific soil conditions (e.g. infertile and acid sandy soils) can result in a complete disappearance of the natural vegetation. A constant lateral (wind erosion) and vertical (percolation) dispersion of metals to the surroundings argues for an immediate restoration of these types of desert-like polluted soils.

For the restoration of such soils, three different strategies can be adopted. These depend upon the nature and degree of the pollution, the desired end use of the area, and the technical and financial means.

Table 1.2. Remediation options for metal-contaminated sites (adapted from Pierzynski *et al.*, 1994 and Martin and Bardos, 1994).

Method	Comments
Ex situ	
Excavation followed by:	
Solidification	Addition of cementing agent to produce a hardened, non-porous, non-leachable material.
Vitrification	Heating to produce a glass-like, non-porous, non- leachable material.
Washing	Chelate or acid extraction.
Leaching	Pile or batch leaching with chelates or acids.
Particle size segregation	Selective removal of finer particle sizes (e.g. clay) that have the highest metal concentrations.
In situ	
Solidification	As described above.
Vitrification	As described above.
Encapsulation	Cover site with impermeable layer.
Attenuation	Dilution with uncontaminated material.
Volatilisation	Promote formation of volatile methylated species (Se, As, Hg).
Vegetative	Promote vegetative growth by providing proper fertility and water availability, reducing metal bioavailability, and/or using metal-tolerant plant species.
Process integration	
Bioventing	Bioremediation and soil venting for the clean-up of petroleum hydrocarbons.
Soil washing	For the clean-up of sites contaminated with both heavy metals and organics.

The most comprehensive solution is digging out the polluted substratum and replacing it with unpolluted soil. Since the polluted upper litter layer strongly inhibits the root development because of its high heavy metal content and its structure, the litter layer should be removed to ensure proper germination of the plants. On a large scale, however, this type of solution is not feasible due to the high costs involved, the problems relating to the safe disposal of the polluted soil and the limited availability of clean soil for replacement.

A second approach could be the *in situ* or *ex situ* purification of the substratum by means of chemical or physico-chemical techniques which 'extract' or 'remove' the metals from the soil (Table 1.3.). The decontaminated substratum can then be reintroduced at the original site, or transported elsewhere. Limited experience with such techniques (Woelders, 1988) has demonstrated that they create new problems, e.g. increasing the mobility (and bioavailability) of the remaining metals in the decontaminated substratum, redistribution of the pollution to other remaining fractions of the process (resins, sludges, etc.), and changes in the physico-chemical characteristics of the treated substratum, reducing its further potential to be used as a normal soil.

A third type of solution is the *in situ* immobilisation of the pollutant by strong metal-immobilising agents and subsequent revegetation of the area. This type of remedial action will reduce the leaching potential of contaminant metals into groundwater and their subsequent transport into the food chain. *In situ* immobilisation is carried out by introducing treatment chemicals into contaminated soils. This could be an economically more realistic and cost-effective alternative, especially for large industrial sites and dumping grounds.

In situ immobilisation will avoid the requirement for excavation of soils or pumping of groundwater. Metals are prevented from migrating through the soil to groundwater and isolated from other chemical and physical processes in the soil and its solution such as hydrolysis, changes in soil pH or varying oxidation-reduction potential which tends to solubilize the metals. Provided the metals remain immobilised, they will not present any adverse environmental or health hazards in their treated state.

Immobilisation therefore reduces metal leaching and bioavailability to plant roots, allowing vegetation to develop which can stabilise the soil. Besides the aesthetic advantage, such vegetation cover provides stability to the soil and lateral wind erosion is prevented (Vangronsveld *et al.*, 1991, 1993).

Most investigations attempting to reduce the availability of metals to plants have concentrated on the maintenance of a high soil pH through liming. In addition, application of zeolites to soil has been shown to greatly reduce plant metal concentrations (Gworek, 1992).

Table 1.3. Chemical or physico-chemical techniques for removing metals from soils.

Method	Comments	Element remediated	References
Electroremediation	An embedded electrode array induces contaminant migration through a combination of electroysis, electro-osmosis and electrophoresis.	Heavy metals	Martin and Bardos, 1994.
Electrokinetic	An electric current passes through the polluted soil, acting as a chelating agent.	Cu, Cr, As	Ribeiro *et al.*, 1995.
Electromigration	Ions in the soil solution move in the direction of the electrode of opposite polarity.	Cu, Cr, As	Ribeiro *et al.*, 1995.
Electroosmosis	The soil solution flows parallel to the electric field being able to be displaced by clean pore water. Increasing the mobility of metals in soil by applying DC electric current across the plant growth media or the growing plants.	Cu, Cr, As, Pb	Ribeiro *et al.*, 1995; Huang *et al.*, 1995.
Electrochemical	Stripping voltammetry with a hydrodynamic sensor, the wall-jet mercury film electrode.	Mo, Ni, Pb, Cu, Cd.	Neto *et al.*, 1995.
Sorbents	Zeolites, glauconite, bentonite	Pb, Cu, Zn, Cd, Mn	Haritonov and Kroik, 1995.

Table 1.3. continued

Steel shots	An iron compound with a great corrosion ability, which becomes oxidised to hematite (α-Fe_2O_3), magnetite ($Fe^{2+}Fe_2^{3+}O_4$) and lepidocrocite (γ-$Fe^{3+}O(OH)$) in the soil.	Cd, Zn, Pb	Rule & Adriano, 1995; Sappin-Didier *et al.*, 1995; Mench *et al.*, 1994, ab; Chlopecka & Adriano, 1995.
Ca-Chelex resin membrane	The chelating resin membrane is embedded in soils as an infinite sink to adsorb soil heavy metals	Cd	Lee and Jiang, 1995.
Chelating agents, complexons	$CaNa_2EDTA$, Trilon B, Unitiol, NN-hydroxy ethylenediamine triacetic acid (HEDTA),Sodium thiosulfate, amino acids are chelating agents which decrease the toxicity of xenobiotics.	Hg, Pb, Cu, Co, Cd, Cr, As, Al, Pb	Tonkopii *et al.*, 1995; Huang *et al.*, 1995.
Soil vapour extraction (SVE)	Drawing clean air through the contaminated soil by means of vacuuming wells, mobilising the contaminants with the gaseous stream.	Volatile contaminants	Garcia-Herruzo *et al.*, 1995.
CO_2 injection	*In-situ* precipitation of calcite	As, Cr	Reddy *et al.*, 1995.
Acid rain	Leaching metals from soils with formic, succinic or sulphuric acid.	Heavy metals	Abrahamsen, 1980; Pierzynski *et al.*, 1994; Nicholson *et al.*, 1995.

An alternative would be to cultivate heavy metal-tolerant species, either leguminous plants associated with specific mycorrhizae (Visser, 1985) or plants physiologically adapted to high tissue concentrations (Geiger *et al.*, 1993).

Smith and Bradshaw (1979) and Johnson *et al.* (1977) have already shown that, on metal-polluted substrata, the use of metal-tolerant plants is the best reclamation technique, giving reasonable prospects of long-term success on these intractable sites. However, when the metal availability to the plants (dependant on soil type, pH, cation exchange capacity, organic matter concentration, speciation of the element) is very high, even metal-tolerant plants are not able to survive universally. Under such conditions, mixing of metal-immobilising additives in the soil to reduce its phytotoxic potential, should be considered (Vangronsveld *et al.*, 1995 b).

A good reclamation procedure should be affordable, reasonably simple, and should fully restore soil fertility and plant quality. Not only can process integration extend the range of treatable contamination problems, it can reduce treatment costs of complex contamination by allowing maximum use of low cost treatments, for example using extensive treatments (such as cropping plants which contain heavy metals or toxic organic compounds) for dealing with the process residues generated by intensive techniques (such as soil washing or incineration). Alternatively extensive technologies can be used to complete the treatment of a partially cleaned site, gradually increasing its range of potential uses, and hence its value in the longer term (Martin and Bardos, 1994).

The use of zeolites as soil amendments does not reduce total metal concentrations of soils, but decreases the bioavailable fraction to a considerable extent. The bioavailable fraction is potentially the most dangerous fraction for uptake by plants and available to animals and human. Reducing metal bioavailability is extremely important in view of the effects of heavy metals in the food chain, where it is desirable to reduce their concentrations at levels far below the phytotoxic threshold values, since phytotoxicity symptoms in crops sometimes appear at metal concentrations many times higher than the guidelines for animal or human consumption. Short-term changes in availability are important, but so too are the long-term changes, which are more difficult to determine (Kabata-Pendias, 1987; Alloway, 1990; Kabata-Pendias and Pendias, 1992).

The residence time for pollutant heavy metals in soils is in the region of hundreds and even thousands of years depending on the element and the type of soil (Kabata-Pendias, 1987). Therefore, the most important questions to be addressed are the changes in the bioavailability of the heavy metals and their distribution in the soil profile during the residual

period and to determine those concentrations of metal pollutants which pose the greatest threat to human health. Regardless of existing threshold values for the metals in soil, it is necessary to investigate the metal behaviour in soil, their potential phytotoxicity, and the feasibility of cost-effective technologies for soil remediation in order to limit soil-plant metal transfer (Kabata-Pendias and Pendias, 1992).

The main points requiring consideration in relation to the behaviour of heavy metals in polluted soils and their impact on the environment are:

(1) the concentrations of heavy metals in polluted soils;

(2) the bioavailability of these heavy metals to crops;

(3) effects of soil properties on the bioavailability of heavy metals and its modification by amendment with synthetic zeolites;

(4) changes in bioavailability during the residual period;

(5) differences between crop species and cultivars in the uptake and accumulation of heavy metals in edible organs;

(6) movement of heavy metals in the soil profile and the potential risk of groundwater pollution (Alloway and Jackson, 1991).

1.5. Reasons for research

There is considerable interest in the development of *in situ* remediation strategies for metal polluted soils that are both durable and robust. Techniques which are based on the incorporation of potential complexing or immobilising agents, which possess a known capacity for long-term and effective metal removal are especially favoured. However, there is a distinct lack of trial data to support the potential benefits of this approach.

Generally, the total metal content of the soil is the primary focus for pollution, but when the food chain is concerned, the mobility and the bioavailability of the metals has to be taken into account. Plant uptake of metals parallels the bioavailable fractions of the metals in soils (Alloway, 1990). Therefore, a logical solution to minimise plant uptake and thus protect the quality of the food chain, or to enhance revegetation, is to render the metals in soil immobile (Kabata-Pendias and Pendias, 1992). There are several ways of immobilising metals in soil. A common technique is to apply dolomitic lime, phosphates, or organic matter residues (Impens *et al.*, 1991). Immobilisation could also be achieved by adding other natural

or synthetic chemical additives that may not produce any detrimental by-product and would enhance the soil ion-exchange capacity.

The application of zeolites 4A and 13X into lead-contaminated soils has been shown to reduce the lead content of plant tissues of several species (Gworek, 1992). Batch equilibration and column testing have indicated that zeolite Valfor G100, when combined with ferrous sulphate, was very effective in immobilising cadmium (Czupyrna *et al.*, 1989). Beringite, a modified alumino-silicate has been shown to fix zinc and cadmium in contaminated soils, and can strongly reduce or eliminate the phytotoxicity of these metals towards the bean (*Phaseolus vulgaris* L.) (Vangronsveld *et al.*, 1990). Other soil additives, such as lime, Thomas phosphate basic slag (TSB), hydrous iron oxides (HIO), hydrous manganese oxides (HMO) and steel shot (ST), are also known to reduce metal mobility in soils (Mench *et al.*, 1994 a, b; Vangronsveld and Clijsters, 1992, etc. - see Table 1.3.).

In situ immobilisation techniques (Tables 1.2. and 1.3.) are designed to reduce the solubility of soil contaminants by enhancing their sorption, precipitation, or complexation. In particular, metals can be fixed by several inorganic ligands, such as iron and manganese oxides, which may be used in conjunction with pH adjustment. The addition of these oxides in soil can reduce the mobility of metals in soil solution (Mench *et al.*, 1994 a).

The effects of acidity of artificial rain on the leaching of plant nutrients have been studied by means of the field lysimeters (Abrahamsen, 1980). Evaluation of the effect of acid precipitation on the amount of plant nutrients can be restricted to consideration for four processes: deposition from the atmosphere, weathering, volatilisation and leaching from the soil. Lysimeter experiments carried out with artificial rain (pH 2.5-5) have revealed a significant leaching of NH_4^+ and NO_3^-.

The pH of the simulated acid rain affects the rate at which metals leach from the soil, illustrating the potential consequences of failing to control emissions of SO_2 and NO_2 which lead to acid rain formation and may have implications for land use changes involving the cessation of liming (Nicholson *et al.*, 1995). Experiments with artificial acidification have shown a reduction in the pool of exchangeable calcium, magnesium and manganese in the soil and an increase levels of protons and aluminium ions. Aluminium ions will be mobilised from soil particles into solution and become toxic both to uptake mechanisms on the fine root hairs and to their partner fungi in mycorrhizal associations with the roots, thereby diminishing their ability to take up essential minerals and water.

Treating metal contaminated soils with additives improving binding of metals to the soil complex can reduce soil phytotoxicity. One class of materials which possess the potential

to immobilise metal pollutants in soils on a long term basis are zeolites. Zeolites, both natural and synthetic, have been shown to reduce the heavy metal content of plant tissues of several species (Gworek, 1992; Mench *et al.*, 1994 a,b; Rebedea and Lepp, 1995; Vangronsveld *et al.*, 1995 ab).

Among the technologies for metal control such as precipitation, oxidation, reduction and coagulation, ion exchange seems to be an attractive method especially when synthetic zeolites are used as exchangers.

Zeolites have a large potential for applications in pollution control and in industrial processes. In this work, the ion exchange capabilities of synthetic zeolites are used to reduce metal phytotoxicity in a range of contaminated soils. Similar technology is widely used for water purification. Here zeolites or other cation exchangers reduce the concentration of metal ions in a solution by a process of ion exchange, replacing undesirable metals such as copper or lead with sodium ions or protons. After use the ion exchanger can be regenerated and the metal ions recovered in a concentrated form.

Zeolites display typical selectivity sequences, and show remarkable affinities for heavy metal cations (Breck, 1974). A basic understanding of the effect of zeolite amendments on the heavy metal content of polluted soils is necessary to evaluate their potential suitability for decreasing metal bioavailability to plants.

The use of synthetic zeolites as amendments for contaminated soils is drawing increased attention due to their low cost of manufacture, thermal stability and high selectivity towards heavy metal ions (Loizidou, 1985). Zeolites are not presently used on a commercial scale for land remediation, although some research has been done regarding this matter. In field trials, the capacity of beringite to fix heavy metals in polluted soils was evaluated. 5% beringite was applied on a sandy soil originating from the vicinity of a zinc smelter, where plant life had been absent for several decades due to metal contamination. A mixture of several grasses and clovers was sown. Plant growth was fully restored and metal specific iso-enzymes were not induced. Beringite immobilised the mobile metal fraction to an extent that the substrate was no longer phytotoxic (Vangronsveld and Clijsters, 1992).

Field experiments have also been carried out with phillipsite synthesised from coal fly ash. The application of phillipsite to polluted soils improved the water capilar capacity and the cation exchange capacity of the soil (Kralova *et al.*, 1994).

The objectives of this study were:

(i) to compare and evaluate the affinities of three synthetic commercial zeolites: P, 4A and Y for a variety of metals;

(ii) through sequential extraction procedures, evaluate the speciation of contaminant metals: copper, cadmium, zinc and lead in soil solutions of three metal-contaminated soils and examine the changes in metal speciation that occur with the addition of zeolite treatments, and to determine any changes over time in the chemical forms of heavy metals in the soil solution and the stability of the zeolite *in situ*;

(iii) to determine the metal concentrations in plants grown on different amended contaminated soils.

CHAPTER 2.
ZEOLITES AND SOIL REMEDIATION.

2.1. Definition and occurrence

A zeolite can be defined as a crystalline aluminosilicate, with a tetrahedral framework structure, enclosing cavities occupied by cations and water molecules, both of which have enough freedom of movement to permit cation exchange and reversible dehydration (Vasant, 1990).

They were discovered in 1756 by Freiherr Axel Fredrick Cronstedt, a Swedish mineralogist, who noted the intumescence properties of stilbite, that is, the way in which crystals visibly lose water when heated. This observation inspired the name "zeolite" which in Greek means "boiling stones". Since that time, nearly 50 natural species of zeolites have been recognised, and more than 100 species having no natural counterparts have been synthesised in the laboratory (Pond and Mumpton, 1984).

Zeolites are formed in a variety of geological environments, such as: saline and alkaline lakes, soils and land surfaces, open hydrologic systems (ground water systems), marine deposits and buried diagenetic or low grade metamorphic deposits (Pond and Mumpton, 1984).

2.2. The structure of zeolites

The zeolite framework consists of $(SiO_4)^{4-}$ and $(AlO_4)^{5-}$ tetrahedra linked together by sharing oxygen atoms at the corners of these alumina and silica tetrahedra. This arrangement reduces the overall oxygen: silicon ratio to 2:1.

The isomorphous substitution of trivalent aluminium for tetravalent silicon in the silicate tetrahedra of zeolite structures creates fixed, negatively charged sites throughout the structure. To maintain electrical neutrality, the negative charges are neutralised by the presence, in the interstices of the structure, of an equivalent number of mobile cations or counterions. Such mobile cations are only loosely bonded in the crystal structure and are free to exchange with cations in solution.

The maximum degree of substitution is when the silicon:aluminium ratio is 1:1. Further substitution of silicon by aluminium is prohibited by the inability of two adjacent linked tetrahedra to both contain aluminium (Loewenstein's Rule, 1954).

The empirical formula of a zeolite may be given as:

$$M_{2/n}O.nAl_2O_3.xSiO_2.\ yH_2O$$

where M represents an alkali or alkaline earth metal cation, n is the cation valency, x is greater than or equal to the number of aluminium atoms present and y is the water content.

Zeolite structures are classified by the presence of specific recurring subunits of structure called "secondary building units" (S.B.U's) which are arrays of silicon (or aluminium) and oxygen tetrahedra (Dyer, 1988). Breck (1974) classified zeolites into 7 groups based on a combination of framework topology with fundamental building units (Fig. 2.1.). These units include the primary building unit of the TO_4 tetrahedron (where T = Si^{4+} or Al^{3+}), secondary building units which consist of both single rings of 4, 5, 6, 8, 10 and 12 tetrahedra and double rings of 4, 6 and 8 tetrahedra, and layer symmetrical polyhedra.

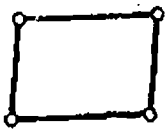
The large cations, coordinated by framework oxygens and water molecules, reside in large cavities in the crystal structure; these cavities and channels may even permit the selective passage of organic molecules. The minimum width of a channel is an approximate measure of the maximum diameter of a molecule which can pass through it (Breck, 1974).

The internal void volume and access is determined by the framework topology and by the presence of non framework species (water, cations, other occlusives). In general, the internal voids consist of: channels - unidirectional or interconnected, or cavity -like voids mutually interconnected through apertures which vary from six to twelve-membered rings of tetrahedra.

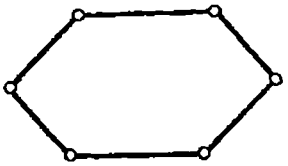
The number of electrochemical equivalents of cations needed to balance the anionic framework charge may also be considerably less than the total number of available cation sites of all kinds. Thus when the cations distribute themselves among the sites so as to minimise the free energy of the system there may be partial occupancy of some or all of the kinds of site available. The distribution equilibria is expected to be a function of temperature, the cationic species present and the state of hydration or dehydration of the zeolite. If all water is removed and other guest molecules are introduced these guest species may also cause a redistribution of some cations, especially if they are polar like ammonia or sulphur dioxide.

Another variable is the silicon/aluminium ratio which determines the anionic charge per unit cell and so fixes the number of cations. As the silicon/aluminium ratio changes, so may the distribution of the cations among the kinds of site present in the zeolite (Barrer, 1978). No zeolites are known that contain more aluminium atoms than silicon; thus, the molecular SiO_2:Al_2O_3 ratio is always equal to or greater than 2:1 (Pond and Mumpton, 1984).

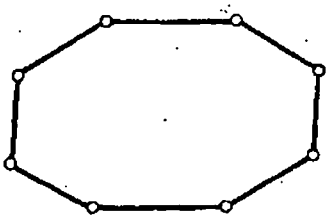
Figure 2.1. The secondary building units (SBU) in zeolite structures according to Dyer (1988). Only the positions of tetrahedral silicons and aluminium are shown.



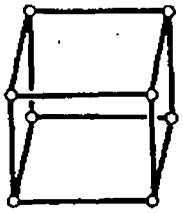
single four ring, S4R



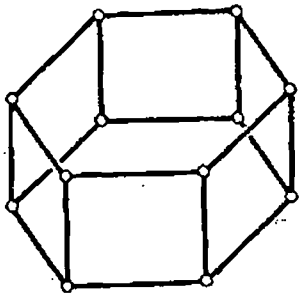
single six ring, S6R



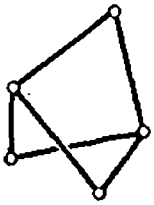
single eight ring, S8R



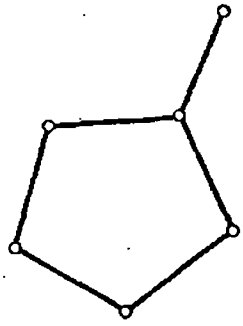
double four ring, D4R



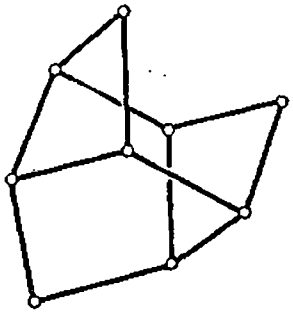
double six ring, D6R



complex 4-1



complex 5-1



complex 4-4-1

2.3. Description of Zeolites Used

2.3.1. Zeolite A

Zeolite A is a synthetic zeolite first reported by Breck *et al.* in 1956. It is synthesised mainly in the sodium form by crystallisation of sodium aluminosilicate hydrogels, although other cationic forms are quite easily obtainable by simple ion-exchange in aqueous solution.The ideal unit cell composition of zeolite NaA is given by:

$$Na_{12}[(AlO_2)_{12}(SiO_2)_{12}].27H_2O.$$

The fundamental building block in zeolite A is the sodalite unit or beta-cage which has a free diameter of approximately 0.66 nm. A β-cage or sodalite unit is made up of 24 tetrahedra (TO_4) linked as six 4-membered rings and eight 6-membered rings in the cagelike fashion of a truncated octahedron (Fig. 2.2.a.). Linking the sodalite units through the single 4-membered rings (S4R) gives the cubic framework structure of the sodalite mineral (Fig. 2.2.b.)(Beagley and Titiloye, 1992).

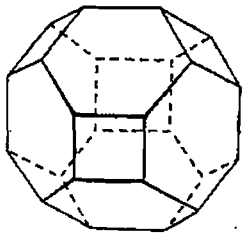
The zeolite A framework is built up in a similar manner to the sodalite mineral, except that the β-cages are linked through "double 4-membered rings" (D4R) to form an octahedral array of β-cages. The presence of the D4R leads to the formation of larger cavities, known as α-cages (Fig. 2.2.c.) which have a cubic structure (Beagley and Titiloye, 1992).

2.3.2. Zeolite P

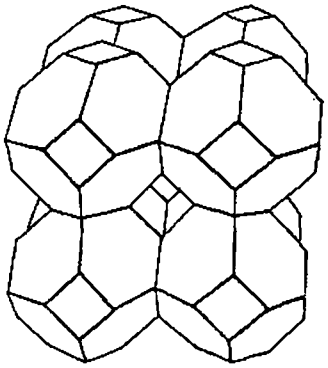
Zeolite P is the synthetic counterpart of the naturally occurring mineral gismodine. Its composition lies in the CaO-Al_2O_3-SiO_2-H_2O chemical system: in nature it is typically $CaO.Al_2O_3.2SiO_2.4H_2O$, though some variation is known to occur. The tetrahedra are cross-linked by the sharing of oxygen atoms, so that O/(Al+ Si) = 2. The resulting framework comprises eight-membered ring channels which pervade the entire structure. In gismodine, calcium occupies the channels to give charge balance (2Al/Ca = 1). Water molecules are also accommodated in this channel network.

Within the synthetic P-group zeolites, chemical compositions are more varied. The general formula for zeolite P is $M_{2/n}O.nAl_2O_3.xSiO_2.yH_2O$, where M represents a metal, and *n* its valence. The x value can vary in the range 2.0 to 5.0, while y varies between 0 and 6.0, depending on the accompanying cation (Atkins *et al.*, 1995). A typical unit cell composition

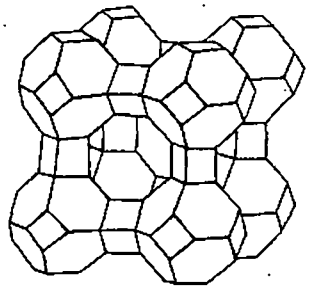
Figure 2.2. Conventional line drawings of framework structures, which ignore the oxygen atoms (each line represents T...T) (Beagley and Titiloye, 1992).



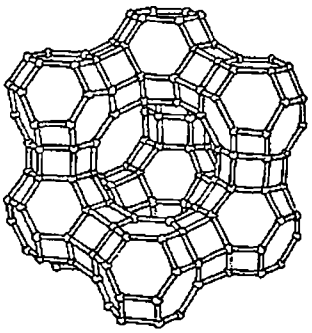
(a) The sodalite unit (β-cage)



(b) Sodalite units linked through S4R to form the cubic framework of sodalite itself



(c) Sodalite units linked through D4R forming the cubic zeolite A framework



(d) Sodalite units linked through D6R forming the faujasite structure (double line represent T...T)

is: $Na_{3.6}Al_{3.6}Si_{12.4}O_{32}.14H_2O$ (Hansen *et al.*, 1990). Two polymorphs of zeolite P are known at room temperature; cubic (P_c) and tetragonal (P_t) (Atkins *et al.*, 1995).

2.3.3. Zeolite Y

Zeolite Y is isostructural with zeolite X and faujasite. It differs from 4A and P in its higher silicon:aluminium ratio. There is a smaller amount of isomorphous substitution of aluminium for silicon into the tetrahedral framework position and this also causes a reduction in the number of water molecules present. It has the general formula:

$$Na_{56}\,(AlO_2)_{56}\,(SiO_2)_{136}\,.\,264\,H_2O$$

with a silicon/aluminium ratio of about 2.4, although a range of 1.2 to 2.4 is possible (Harjula *et al.*, 1993).

The structure of faujasite is closely related to the A type structure, except that the sodalite units are linked through double 6-membered rings (D6R) in a diamond like tetrahedral array of β-cages, utilising four of the eight S6R faces of each β-cage (Fig. 2.2.d.). The synthetic zeolites " Linde" X and Y have the largest cavities and cavity entrances of the strictly aluminium- silicate zeolites (Sherry, 1968).

Zeolites with larger cavities are known which belong to the pentasil group and include zeolites ZSM-5, ZSM-11, ZSM-48. The SiO_2/Al_2O_3 ratio for zeolite ZSM-5 ranges between 25-1000. Because of their large cavities, these zeolites are used for accommodating complexes and large organic molecules. ZSM-5 can accommodate large ions such as molybdenum, gallium, vanadium and titanium, and is an excellent catalyst for the oxidation of benzene to phenol and for propylene oligomerization (Barrer, 1978; Townsend, 1979).

2.3.4. Berengite

Berengite is a calcined micaceous schist that originates from the fluidized bed burning of coal refuse from the former coal mine of Beringen in the NE of Belgium. Berengite is made from altered schists of the Paleozoic period (Westphalien deposits), which are pulverized and then roasted in a fluidbed oven at 800°C. During the heating process, the schists are broken down and partly recrystallized. Using air suction, particles with an average diameter between 0.02 and 0.2 mm are separated from the others. Berengite also contains elements from other minerals which are present in the schists:

calcite ($CaCO_3$), dolomite ($Ca/MgCO_3$), anhydrite ($CaSO_4$), siderite ($FeCO_3$) and pyrite (FeS_2). A specific surface area of about 20 m^2/g was measured by De Boodt, 1991. Table 2.1. shows a typical chemical composition of berengite (De Boodt, 1991). SiO_2 and Al_2O_3 are the main fractions which comprise 82% of the whole structure (Wessolek and Fahrenhorst, 1994).

Table 2.1. Chemical composition of Berengite (De Boodt, 1991).

Element	Percent	Element	Concentration (mg/kg)
SiO_2	52	Mg	1100
Al_2O_3	30	Cu	120
CaO	3.5	Zn	630
MgO	1.5	Cd	9
K_2O	2.7	Co	98
Na_2O	0.6	Ni	123
Fe_2O_3	4.7	Pb	203

2.4. Zeolite Properties

Each zeolite species has a unique crystal structure, and hence, its own set of physical and chemical properties:

(a) Adsorption properties:

This is a fundamental property of zeolites. The large cavities and entry channels of zeolites are generally filled with water molecules that form hydration spheres around exchangeable cations (Pond and Mumpton, 1984). The location of the cations and water of hydration in any zeolite framework are functions of temperature, water content, cation type and silicon:aluminium ratio (Dyer, 1988).

To activate zeolites for molecular sieves or cracking catalysts, it is necessary to remove as much as possible of this water. If the water is removed, usually by heating the zeolite for several hours or overnight at 350-400° C, molecules small enough to fit

through the entry channels are readily adsorbed on the inner surfaces of the dehydrated central cavities. Molecules too large to fit through the entry channels are excluded and pass around the outside of the zeolite particle, giving rise to the well-known "molecular sieving" of most crystalline zeolites (Pond and Mumpton, 1984).

Molecular sieves have the ability to undergo dehydration with little or no change in crystal structure. The dehydrated crystals are honeycombed with regularly spaced cavities interlaced by channels of molecular dimensions which offer a very high surface area for the adsorption of foreign molecules.

The properties of molecular sieves as adsorbents which distinguish them from non-zeolitic adsorbents are the relatively strong coulomb fields generated by the adsorption surface and the uniform pore size; the pore size is controlled by the associated cation (Breck, 1974).

The "effective pore size" is defined by the largest molecule able to pass through the constriction. Zeolites with a low aluminium content and consequently few cations have a low water content, so that their frameworks have hydrophobic tendencies, in sharp contrast to the normally highly hydrophilic character of most other known zeolites (Dyer, 1988).

(b) Cation-exchange properties:

The exchangeable cations of a zeolite are only loosely held in the tetrahedral framework and can be removed or exchanged easily by washing with a strong solution of another cation. As such, crystalline zeolites are some of the most effective cation exchangers known. The number of cations available for exchange is given by the C.E.C. The cation-exchange capacity (C.E.C.) is a measure of the number of counterions present per unit weight or volume of the zeolite. The cation-exchange capacity is primarily a function of the degree of aluminium substitution for silicon in the framework structure: the greater the substitution, the greater the deficiency of positive charge and the greater the number of alkali or alkaline earth cations required for electrical neutrality. The framework of a crystalline zeolite dictates its selectivity towards competing ions by size preference.

(c) Extensive properties:

Sedimentary zeolite ores are generally soft, friable, and light weight, having bulk densities of 1.2 to 1.8 g/cm^3 (Barrer, 1978).

2.5. Zeolite applications

Ion-exchange is an intrinsic property of zeolites. As a consequence the phenomenon has given rise to the potential use of zeolites as cation scavengers in contaminated land remediation and wastewater purification techniques. There are a wide range of natural zeolite minerals which may be applied to polluted soils, but their properties are fixed and may not be manipulated. Synthetic zeolites are familiar industrial materials, whose synthesis may be manipulated to generate products with a wide range of chemical properties which relate to their abilities to trap and bind metals in solution.

The use of natural and synthetic zeolites for pollution control and remediation depends on the ion-exchange capability of the alkali metals within the zeolite structure and the selectivity of the zeolite in particular towards toxic metals. The possibility of zeolites for *in situ* remediation techniques will also depend on the long term stability of the metal-zeolite complex and permanently reducing metal bioavailability. As a result of their high selectivity for many metals, especially some of the more toxic ones such as lead and cadmium, zeolites may provide an alternative media for metal immobilisation.

The first practical use of zeolites probably occurred about 2000 years ago when natural zeolite rock was quarried for use as building stone although zeolites were not recognised as a new mineral species until 200 years ago. The first physico-chemical property of zeolites which had an application (cation exchange) was investigated by Eichhorn about 100 years ago. This led to the development of synthetic, amorphous aluminosilicates as commercial cation exchange materials (permutites) in the early 20^{th} century, which were primarily used in water softening. The first application of dehydrated zeolites as molecular sieves in the separation of gas mixtures was demonstrated by Barrer in 1945 utilising the zeolite mineral chabazite. Synthetic zeolites were first introduced and utilised commercially as molecular sieve adsorbents in 1954 (Townsend, 1979).

There are more than 50 distinct species of zeolite that occur in nature. However, only seven, mordenite, clinoptilolite, ferrierite, chabazite, erionite, phillipsite, and analcime, occur in sufficient quantity to be considered as viable mineral resources (Vaughan, 1978). The availability of large natural deposits of these zeolites which can be mined at low cost also make the use of these minerals attractive. Large deposits of natural zeolites may provide cost-effective alternatives for treatment of soil and water contaminated with heavy metals and radionuclides. Some of the environmental

applications of natural zeolites include: (a) the use as a soil amendment for reduction of fertiliser use and pollution from agricultural lands; (b) sorbent barriers as liners for disposal sites containing heavy metals or radionuclides; (c) drinking water treatment; (d) sewage treatment; (e) sorption of organic toxins from air; and (f) use in solar panels for alternative energy sources (Leppert, 1991).

The potential successful applications of zeolites in many fields including those of agriculture, animal dietary, fertiliser, petroleum industries and waste treatment have been extensively reported in the literature and have received world-wide interest. Nevertheless, zeolites are not widely used as commercial ion-exchange materials, because of their susceptibility to acid attack.

There are four main areas where zeolites are finding direct application as ion-exchangers: in detergents, in ammonia/ammonium removal from freshwater effluent, in radioisotope removal from spent fuel effluent and in agriculture. By far the most important of these applications is in detergents, where zeolites are employed as a water softeners, partially replacing tripolyphosphate builders. The introduction of synthetic zeolites into laundry detergents to replace environmentally undesirable phosphate has made zeolite a household word.

Zeolite A has the advantage of being a "maximum aluminium" structure containing the maximum possible proportion of aluminium to silicon, so that its capacity for taking up calcium ions from aqueous solution is intrinsically greater than those of zeolite Y and P which generally contain a lower proportion of aluminium (or a higher silicon:aluminium ratio).

The synthesis of a novel zeolite P (maximum aluminium zeolite P, or zeolite MAP) having an especially low silicon to aluminium ratio, not greater than 1.33 and preferably not lower than 1.15 has been described (Araya, 1995). This zeolite is demonstrated to be a more efficient detergency builder than zeolite 4A (Chapple, 1993).

One of the earliest applications of synthetic zeolites for ion-exchange was in the removal and purification of caesium and strontium radio-isotopes for the nuclear energy industry. The use of zeolites in this field has concentrated on three important areas: (1) removal of ^{137}Cs from high-level radioactive wastes; (2) decontamination of low- and intermediate-level wastes; and (3) fixation of radioactive wastes for long-term storage. They offer significant advantages over organic resin ion exchangers because of their stability in the presence of ionising radiation, low solubility and dimensional stability (Townsend, 1979).

Natural zeolites (especially clinoptilolite) are used in the removal of ammonia and ammonium ions from freshwater effluent. Nishita and Haug (1972) conducted studies indicating that addition of clinoptilolite to soil contaminated with ^{90}Sr decreased strontium uptake by plants. Clinoptilolite displays a strong affinity for caesium, lead and cadmium and can selectively absorb some radionuclides and organic compounds (Leppert, 1991). Apart from their use in radio-isotope removal, a major factor which has been taken into consideration in choosing zeolites, rather than other exchangers, for these four applications is cost, zeolites being cheaper than resins. Cost is not a major consideration for radio-isotope removal, it is the high resistance of zeolites to thermal and radiation damage which makes them an attractive option (Townsend, 1984).

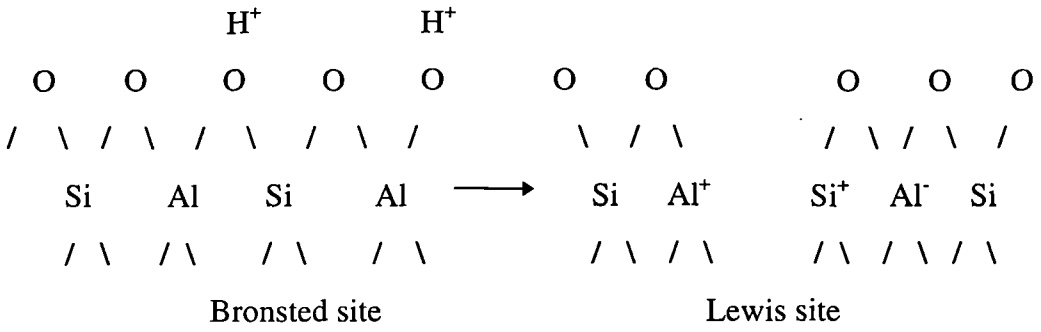
Work has also been conducted on using zeolites as soil amendments. A soil amendment is defined as a substance that aids plant growth indirectly by improving the condition of the soil. Soil amendments should not be confused with plant nutrients, such as nitrogen, that are used directly by the plants. Natural zeolites have been used in Japan as soil amendments for years because of their ion-exchange and water retention capabilities (Hoye *et al.*, 1987). As soil amendments, zeolites appear to retain moisture longer after irrigation and to improve the cation-exchange capacity of the soils (Townsend, 1979).

Zeolites have been used to improve soil fertility. Slow-release fertilisers enriched with ammonium ions have been developed, which also act to improve the buffering capacity of soils. The pronounced selectivity of clinoptilolite for large cations, such as ammonium and potassium has been exploited for the preparation of chemical fertilisers that offer a slower release of these elements to the soil and thus a more efficient uptake by plants. In rice fields, where nitrogen efficiencies of less than 50% are not uncommon, Minato (1975) reported a 63% improvement in the amount of nitrogen available in a highly permeable paddy soil four weeks after about 40 ton/acre of clinoptilolite-rich tuff had been added along with a standard fertiliser. Natural clinoptilolite added to soil in conjunction with urea reduced the growth suppression that normally occurs when urea is added alone. The presence of zeolites also resulted in less nitrate-nitrogen being leached from the soil. The ammonium-exchanged clinoptilolite acted as a reservoir for ammonium that was produced by the decomposition of the urea, and thereby prevented both ammonium and nitrate toxicity by disrupting the bacterial nitrification process. The ability of clinoptilolite to sorb excess moisture represents an attractive addition to chemical fertilisers to prevent caking and hardening during storage.

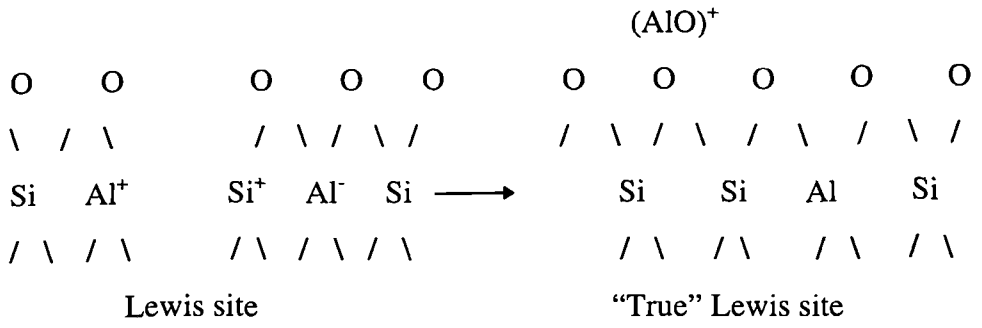
Zeolites exhibit high selectivity for various heavy metals and are considered suitable for the removal of precious and semi-precious metals as well as the removal of heavy metals from industrial and processing waste waters. Blanchard *et al.* (1984) investigated the removal of ammonium and heavy metal ions from drinking water and found that the selectivity of the sodium-exchanged clinoptilolite decreases in the order $Pb^{2+} > NH_4^+ > Cu^{2+}$, $Cd^{2+} > Zn^{2+}$, $Co^{2+} > Ni^{2+} > Hg^{2+}$. Similar observations that clinoptilolite exhibits high levels of exchange for lead and cadmium have been reported (Semmens and Martin, 1988; Loizidou and Townsend, 1987).

In addition to the above, applications are being developed for sedimentary zeolites as fillers in the paper industry, as lightweight aggregates in construction, as ion-exchangers in the purification of water and municipal sewage effluent, in the separation of oxygen and nitrogen from air, as reforming petroleum catalysts, as acid-resistant adsorbents in gas drying and purification and in the removal of nitrogen compounds from the blood of kidney patients (Pond and Mumpton, 1984). A rather exotic application of synthetic zeolites is in the improvement of coffee aroma by removal of pungent volatiles using zeolites 4A and 5A (Sakano *et al.*, 1996).

A major application of zeolites is for cracking long-chain hydrocarbons of crude oil to useful C_1 - C_6 fractions (Dyer, 1988). Acidic silica alumina catalysts have been developed for the cracking reaction. The activity required is based upon the production of Bronsted sites arising from the creation of "hydroxyls" within the zeolite pore structure. Ideally the "protonated" form contains hydroxyls which are protons associated with negatively charged framework oxygens linked into alumina tetrahedra, i.e. Bronsted sites are created:



The Lewis sites in turn are unstable, especially in the continued presence of water vapour and an annealing process stabilises the structure. This produces the "true" Lewis sites by ejecting aluminium species from the framework, i.e.:



The uniqueness of zeolites as cracking catalysts lies in the high density of these active sites coupled with the zeolite inherent stability and amenability to regeneration (Dyer, 1988).

With such a variety of interesting ion-exchange properties, it is unusual that the application of zeolites as ion-exchangers is not more widespread. There are two main reasons. First, many zeolites are relatively unstable even in mildly acid solution (such as pH 4 to 5). To attain a high resistance to acid attack, a material with a high silicon:aluminium ratio is needed (for example, clinoptilolite or mordenite) - the higher the value of this ratio, the lower the exchange capacity of the zeolite. Second, there are problems in obtaining a satisfactory rate of exchange for column operation. The rate at which a given ion exchanges into discrete crystallites of the zeolite frequently bears comparison with exchange rates for the same ion into a typical macroreticular resin at ambient temperature. In order to obtain an acceptable throughput rate, it is necessary to pelletise the zeolite. This results in a drop in the exchange rate to a value which is often far below that found for a macroreticular resin of comparable bed size (Townsend, 1984).

Nevertheless, the ability of zeolites to exchange cations makes them attractive alternatives for removing undesirable heavy metal ions from polluted soils. The usefulness of synthetic zeolites for pollution control applications depends primarily on their ion-exchange capabilities and their porous, three-dimensional framework structure.

Previous studies have shown that the addition of synthetic zeolite pellets to soils contaminated with cadmium significantly reduced the concentrations of cadmium in the roots and shoots of a range of crop plants, including lettuce *(Lactuca sativa)*, oats *(Avena sativa)* and ryegrass *(Lolium perenne)*.

Use of synthetic foyazite group zeolites 4A and 13X, at application rates of 1% soil weight, caused reductions in metal concentrations in monocotyledonous (grasses and

oats) and dicotyledonous (lettuce and table beets) plants grown in contaminated soils amended with these zeolites. For ryegrass, the addition of zeolites to the soil resulted in an approximately 74% reduction of cadmium concentration in the leaves and 52% reduction in the roots, whereas for lettuce leaves the cadmium concentration decreased by 86%. The lead content in lettuce leaves was 49-73% lower; for grasses, these reductions were 47-77%, for the aboveground parts of oats 58-68%, for beet leaves 62%, and for beet roots 26-83% (Gworek, 1992).

Synthetic zeolites 3A, 4A, 5A and 13X have also been introduced into copper contaminated soils at levels of 1, 2 and 3% by weight in relation to the soil mass. The copper content of lettuce grown in the contaminated soil decreased in the presence of zeolites by 29-77%, in grass by 41-78%, in oats by 45-64% and in beets by 21-41%, as compared to the control (Gworek, 1993).

The application of the synthetic zeolites is gaining importance in cleaning industrial sewage sludges of toxic amounts of heavy metals. Investigations have shown that sewage sludge incubated with synthetic zeolites X and 4A resulted in a reduction in the metal content (70% lead, 57% copper, 53.5% nickel, 67.5% zinc, and 61% cadmium) of the sludge studied (Gworek, 1992).

The chemical modification capabilities of the synthetic zeolites in order to provide specific properties may provide a cost-effective alternative for the treatment of heavy metal contaminated soil and water. The application of synthetic zeolites in industrial pollution control is becoming important and the level of technical effort is increasingly expanding (Kesraoui-Ouki *et al.*, 1994).

CHAPTER 3.
INVESTIGATION OF ION EXCHANGE EQUILIBRIA IN THE METAL - ZEOLITE SYSTEM.

3.1. Introduction

Zeolites are a potential source of exchangeable cations and ion-exchange is undoubtedly one of their most important properties. Isomorphous replacement of silicon by aluminium within a zeolite lattice gives rise to an excess negative charge on the framework. Electroneutrality within the zeolite is maintained by the presence of cations which reside in the cavities and channels of the zeolite. These cations are usually mobile and may be exchanged with other similarly charged cations, or possibly with cations of different nature and valency in aqueous solutions under favourable conditions (Dyer, 1988).

Assuming that the zeolite is in equilibria with the aqueous phase it is in contact with, three main properties are important in ion-exchange applications. They are: exchange kinetics, ion-exchange capacity and cation selectivity.

Ion-exchange kinetics concerns the time required for the counter-ion to travel to the exchange site and displace a cation in the structure. The ion-exchange capacity refers to the number of milli-equivalents (meq) of a given cation per gram of zeolite that can be retained in a maximally exchanged zeolite. This is a function of the silica to alumina mole ratio and cationic form. The cation selectivity refers to the preference order of a zeolite for cations, based on the energetics of the distance between anionic sites, cationic radii, and cationic hydration energies (Kesraoui-Ouki *et al.*, 1994).

Ion-exchange can be between cations of the same or different charges and can be expressed according to equation (1),

$$z_A B_{(c)}^{z^+B} + z_B A_{(s)}^{z^+A} \Leftrightarrow z_A B_{(s)}^{z^+B} + z_B A_{(c)}^{z^+A} \qquad (1)$$

where $z_{A,B}$ represents the valencies of the cations A and B, respectively, and the subscripts (s) and (c) refer to the solution phase and zeolite crystal phase respectively. The ion A, initially in solution, is frequently referred to as the counter ion. If cation B, initially in the zeolite, is in contact with a solution containing cation A, then providing conditions are favourable and the

zeolite has a preference for the counter ion A, ion-exchange will take place and can be described by equation (1).

The exchange equilibria for these ions can be characterised by an ion-exchange isotherm, which is a plot of the concentration of an exchanging ion in solution against the concentration of the same ion in the exchanger zeolite at constant temperature and constant solution normality. In general, the isotherm is plotted in terms of the equivalent fraction of the entering ion in solution (A_s) against that in the zeolite (A_c). The equivalent fraction of A_s in solution is given by:

$$A_S = \frac{z_A m_S^A}{z_A m_S^A + z_B m_S^B} \qquad (2)$$

where m_s^A and m_s^B are the concentrations (mol. dm^{-3}) of the ions A and B, respectively, in the equilibrium solution, also $(A_c + B_c) = 1$ and $(A_s + B_s) = 1$. A_c may be similarly expressed, but it is more conveniently expressed in terms of the ion-exchange capacity of the exchanger, equation (3).

$$A_C = \frac{z_A M_A}{z_A M_A + z_B M_B} = \frac{z_A M_A}{Q} \qquad (3)$$

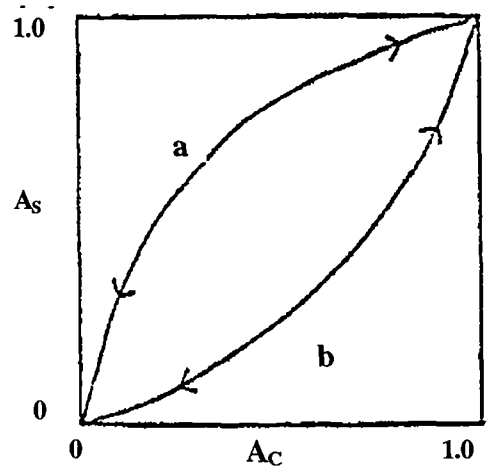
where $M_{A,B}$ is the concentration (mol. Kg^{-1}) of the ions A and B respectively in the zeolite, and Q is the ion-exchange capacity expressed as the number of charges per 100 g of zeolite after equilibration over a saturated salt solution (eq/100g).

Experimentally, ion-exchange isotherms are obtained by equilibrating solutions of constant total solution normality, but having varying proportions of the two exchanging cations, with known sample weights of the homoionic zeolite of interest. The equilibrium composition of both crystal and solution phase are generally determined by analysis of the equilibrated solutions, or by direct analysis of the zeolite.

Different types of ion-exchange are possible in zeolites which depend on many parameters, e.g. selectivity. The ion-exchange mechanisms are represented by the types of isotherm curves shown in Figs. 3.1.1. - 3.1.3. and represent:

Figure 3.1.1. Isotherm Types a and b.

(A_C = equivalent fraction of exchanging ion in the crystal.
A_S = equivalent fraction of exchanging ion in solution).

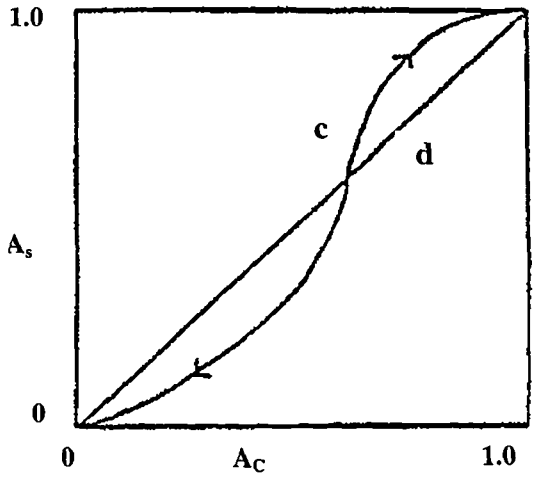


(a) The exchanger is selective for the outgoing ion B, over the entire range of zeolite compositions (Fig. 3.1.1.).

(b) The exchanger is selective for the ingoing ion A, over the entire range of zeolite compositions (Fig. 3.1.1.).

Figure 3.1.2. Isotherm Types c and d.

(for legend see Fig. 3.1.1.)

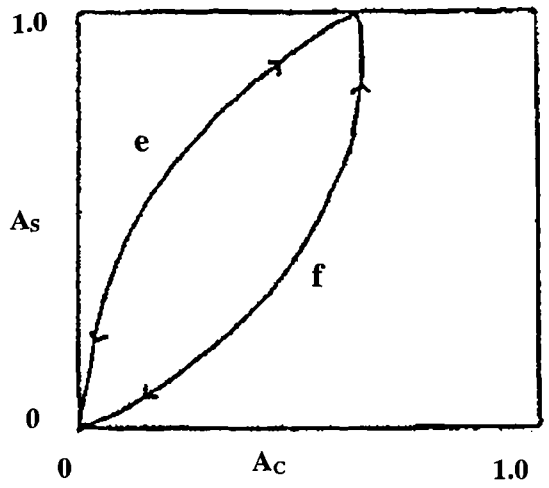


(c) There is a definite concentration dependence at which the exchanger will be selective for ion A (Fig. 3.1.2.).

(d) This shows that the zeolite has no preference for either ion A or B over the whole isotherm (Fig. 3.1.2.).

Figure 3.1.3. Isotherm Types e and f.

(for legend see Fig. 3.1.1.).



(e) Exchange does not go to completion although the outgoing ion B is initially preferred (Fig. 3.1.3.).

(f) Exchange does not go to completion although the ingoing ion A is initially preferred (Fig. 3.1.3.) (Amin, 1991).

3.2. Factors affecting ion-exchange

The ion-exchange properties of zeolites depend on the structure of their crystal lattices and the distribution of cations over various possible positions in channels and cavities of diverse crystal structures.

The cation exchange behaviour of zeolites also depends upon: (1) the nature of the cation species; (2) the temperature; (3) the concentration of the cation species in solution; (4) the anion species associated with the cation in solution; (5) the solvent; and (6) the structural characteristics of the particular zeolite (Breck, 1974).

Ion-exchange may occur in zeolites without significant changes to the zeolite structure as a result of the rigid three-dimensional framework which does not allow appreciable swelling to occur during the ion-exchange process. In zeolites where the internal void space consists of ion exchange sites accessible through larger pores as well as sites accessible only through smaller pores, total ion-exchange may only be available to the smallest ions and only partially available to the larger ions (Dyer, 1988). A further difficulty is encountered in obtaining fully exchanged zeolites with divalent ions such as calcium or cadmium. The type of coordination complex formed between the metal and zeolite in the

small and big cavities hinders the coordination ability of the divalent ions. Because the volume of lattice cavities and channels in zeolites is limited, completeness of sorption is, to a considerable extent, determined by the size of cations themselves and the energy of their hydration (Maes and Cremers, 1974).

The zeolite ion-exchange affinity sequence is often found to be in accord with the hydrated ionic radius. A zeolite generally favours the least hydrated ion, while the solution phase favours the most highly hydrated ion and therefore water molecules in solution are competing with the zeolite for attraction of the cation. The smaller the radius of the hydrated ion, the more preferential the sorption (Naumova *et al.*, 1994). Additionally, if the pore volume of the zeolite is smaller, then the degree of hydration possible for ions within the zeolite will be limited (Barrer and Townsend, 1975).

The degree of cation hydration increases with a decrease in the Pauling radii of divalent cations. The sequence of the ionic radii for the metals studied here are: Cu (0.72Å) < Zn (0.74Å) < Cd (0.97Å) < Pb (1.20Å) (Elfattah and Wada, 1981). Large, fully hydrated ions, such as cadmium or lead are preferred by "weak" field zeolites, i.e. those with lower aluminium content and thus lower framework charge densities, such as zeolite Y.

In addition to the ion hydration characteristic and ionic radius, exchange is also governed by the coordination ability of the transition metal ion. The direct coordination of the divalent ion with the oxygen framework can be compared with an inner sphere co-ordination complex, the interposition of water molecules giving rise to an outer sphere complex with respect to the zeolite framework (Maes and Cremers, 1974).

Each zeolite can be considered as a different and unusual ligand which can impact the coordination shell and chemistry of the cations through unusual electrostatic forces, varying topologies, and multiple types of cation sittings. The activity of transition metals in zeolites varies directly with the level of exchange, which is often achieved by using high alumina (low silicon / aluminium) zeolites. However, as more cation sites are added with increasing levels of alumina, the alumina centres get closer to one another. The high aluminium content not only provides a greater number of exchangeable cations but also permits positioning of two cations fairly close to one another and can be used to demonstrate cooperative effects (Armor, 1994).

When zeolites come into contact with acidic solutions, proton exchange will occur. Therefore, the pH of the exchanging solution is particularly important, because acid solutions can partially hydrolyse and dealuminate zeolites (especially if the zeolite has a high aluminium content, such as zeolite X) (Fletcher and Townsend, 1980). Zeolites which are used for ion-

exchange in acid media gradually lose their sorption properties. Therefore, the employment of zeolites at optimum pH values of 5 to 8 is recommended (Naumova *et al.*, 1994).

With the exception of some monovalent cations (e.g. caesium, potassium, lithium) and divalent cations (e.g. barium, strontium), most metals form more or less insoluble oxides and hydroxides as solution pH increases (Table 3.1.).

Table 3.1. Solubility Products of Some Metal Hydroxides (Amin, 1991).

Metal hydroxide	Ca^{2+}	Mg^{2+}	Cd^{2+}	Pb^{2+}	Zn^{2+}	Cu^{2+}
Solubility product (mol^3/l^3)	10^{-6}	10^{-11}	10^{-14}	10^{-16}	10^{-17}	10^{-20}

During the loading of aluminosilicate zeolites with heavy metal cations by ion-exchange, metal hydroxides may precipitate. The reason for the formation of hydroxides during the ion-exchange process is the alkaline reaction of the aluminosilicates in aqueous solution. Before di- or trivalent cations enter the crystal, the smaller hydronium ions resulting from the hydrolysis of water, partially exchange with the sodium cations of the zeolite. Thereby, the hydroxyl ions of the sodium hydroxide react with the heavy metal cations to form partially soluble solids which precipitate at the crystal surface. In dilute salt solutions (< 0.05M) the hydroxides may dissolve under the action of the re-exchanged hydronium ions. In more concentrated solutions (> 0.05M) the precipitates are stable due to the high concentration of the di- or trivalent cations in the solution after reaching equilibrium (Wark *et al.*, 1994).

The precipitation of surface deposits increases as the solubility product of the metal hydroxide decreases. Surface deposits of insoluble metal hydroxides have been noted on zeolites during ion-exchange processes. To overcome the precipitation of transition metal oxides and hydroxides, the exchanging solution is buffered at an appropriate pH.

3.3. Experimental Ion-Exchange Studies.

Three synthetic zeolites P, 4A and Y were obtained from Crosfield Chemicals, Warrington as fine powders and were supplied in the sodium form. Berengite was obtained from Limburgs University, Belgium. All chemicals used for the analysis and ion-exchange reactions were of analytical-reagent grade.

3.3.1. Preparation of reagents

i) 0.1 M $NaNO_3$. $NaNO_3$ (8.499 g, A.R.) was dissolved in deionised water and transferred to a 1 dm^3 volumetric flask and diluted to volume.

ii) A saturated sodium sulphate solution was prepared by dissolving Na_2SO_4 (142.07 g, A.R.) in a minimum volume of deionised water.

iii) Heavy metal solutions (0.1M) were prepared by dissolving appropriate amounts of each metal nitrate in deionised water (1 dm^3): $Cu(NO_3)_2$ (9.3700 g, A.R.), $Cd(NO_3)_2.4H_2O$ (15.4235 g, A.R.), $Zn(NO_3)_2.6H_2O$ (14.8735 g, A.R.) and $Pb(NO_3)_2$ (16.5600 g, A.R.).

3.3.2. Identification of zeolites.

3.3.2.a. Thermal analysis

Differential thermogravimetric analysis (DTG) was carried out on a Standard Perkin Elmer TGA 7. A sample of zeolite (0.15 mg), previously dried at 60°C, was heated at a rate of 5°C per minute over a temperature range of 20°C to 750°C, and the weight loss, and energy differential recorded. The thermogravimetric curves of all zeolites reveal an endothermic weight loss due to dehydration. This process is complete at about 300°C (Fig. 3.2. a., b., c, d.). The weight losses observed for each zeolite agree with the theoretical values determined from the unit cell formulae (Chapter 2) (Table 3.2.).

Crystallinities and phase purities were checked by X-ray diffraction and SEM.

Table 3.2. Percentage of water in the zeolite samples.

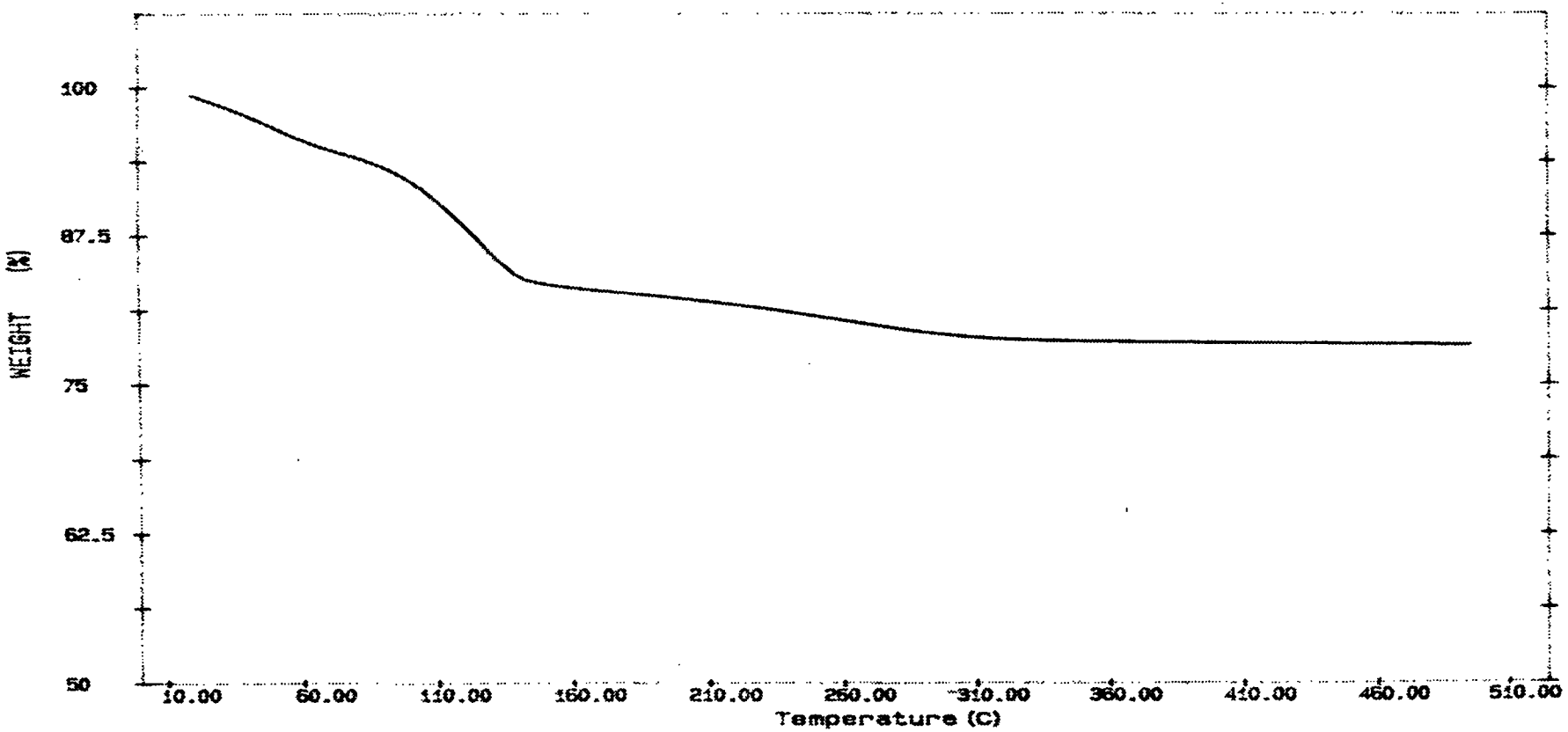
% H_20	P	4A	Y	Berengite
Theoretical	19.4	22.2	25.0	5.0
Experimental	20.0	24.0	26.3	2.5

3.3.2.b. X-Ray Diffraction

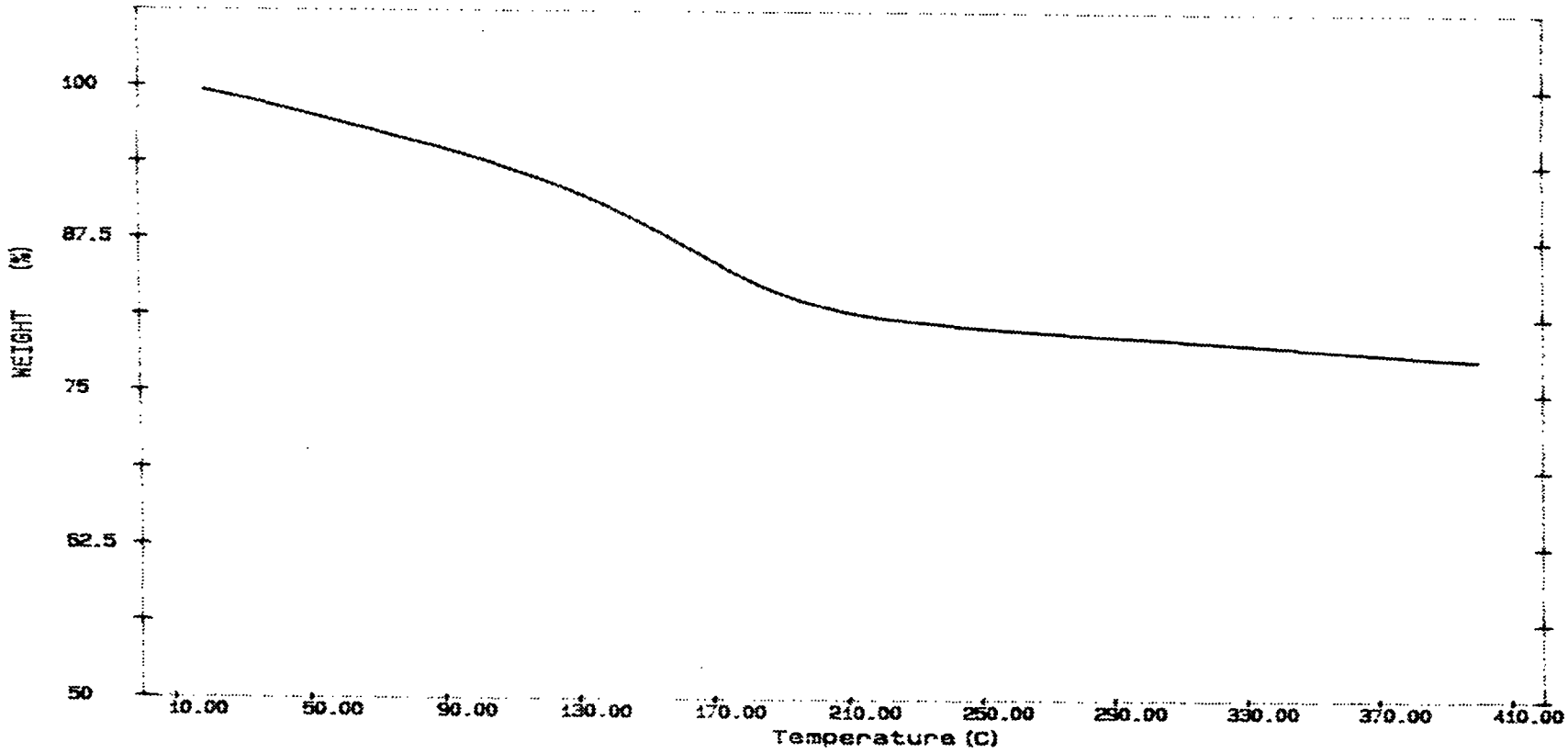
In order to check the crystallinity and to confirm the identity of the zeolites, samples of the original materials were examined by powder X-ray diffraction. X-ray irradiation of

Figure 3.2. TGA for zeolite samples.

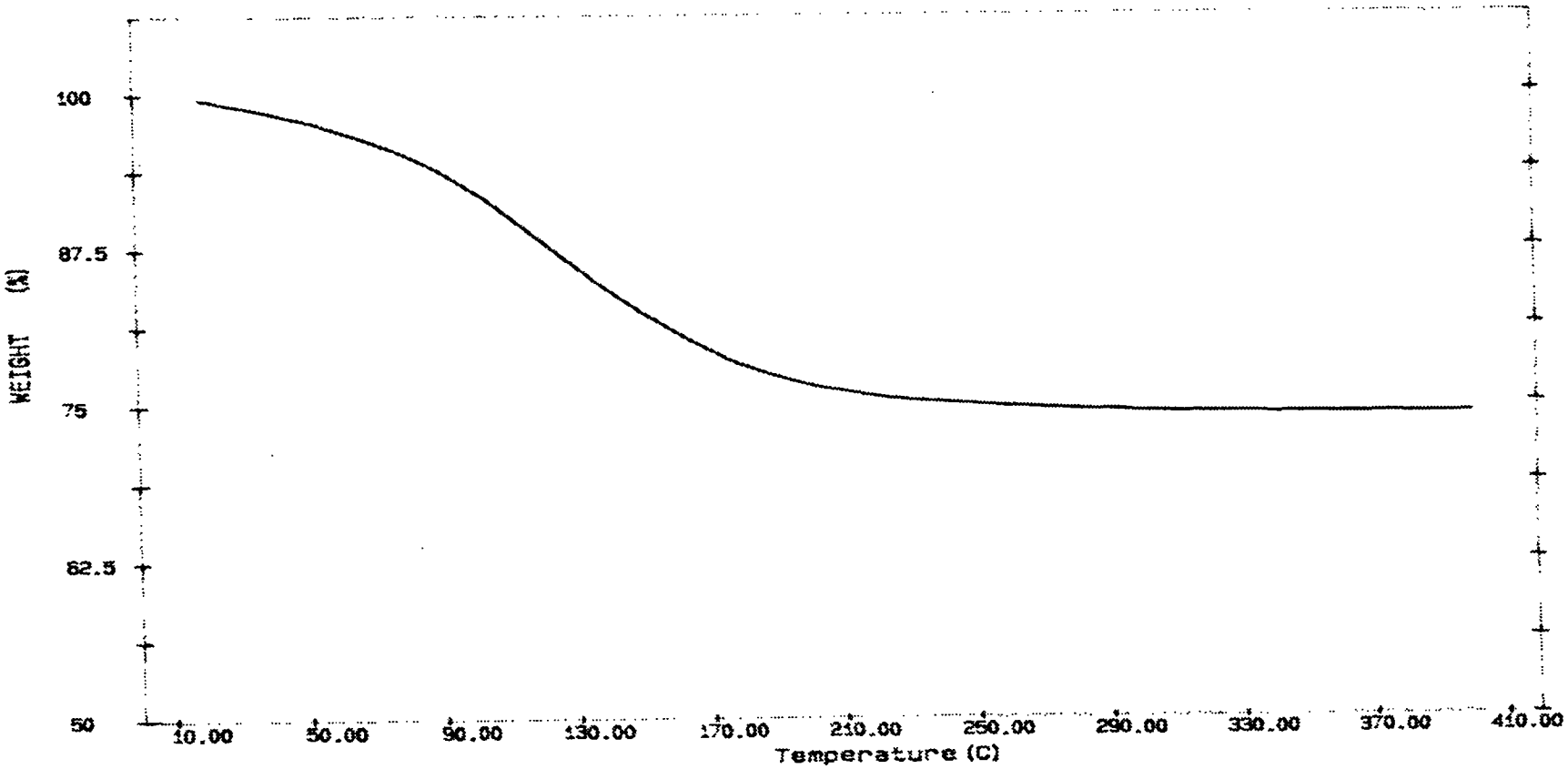
a.Zeolite P



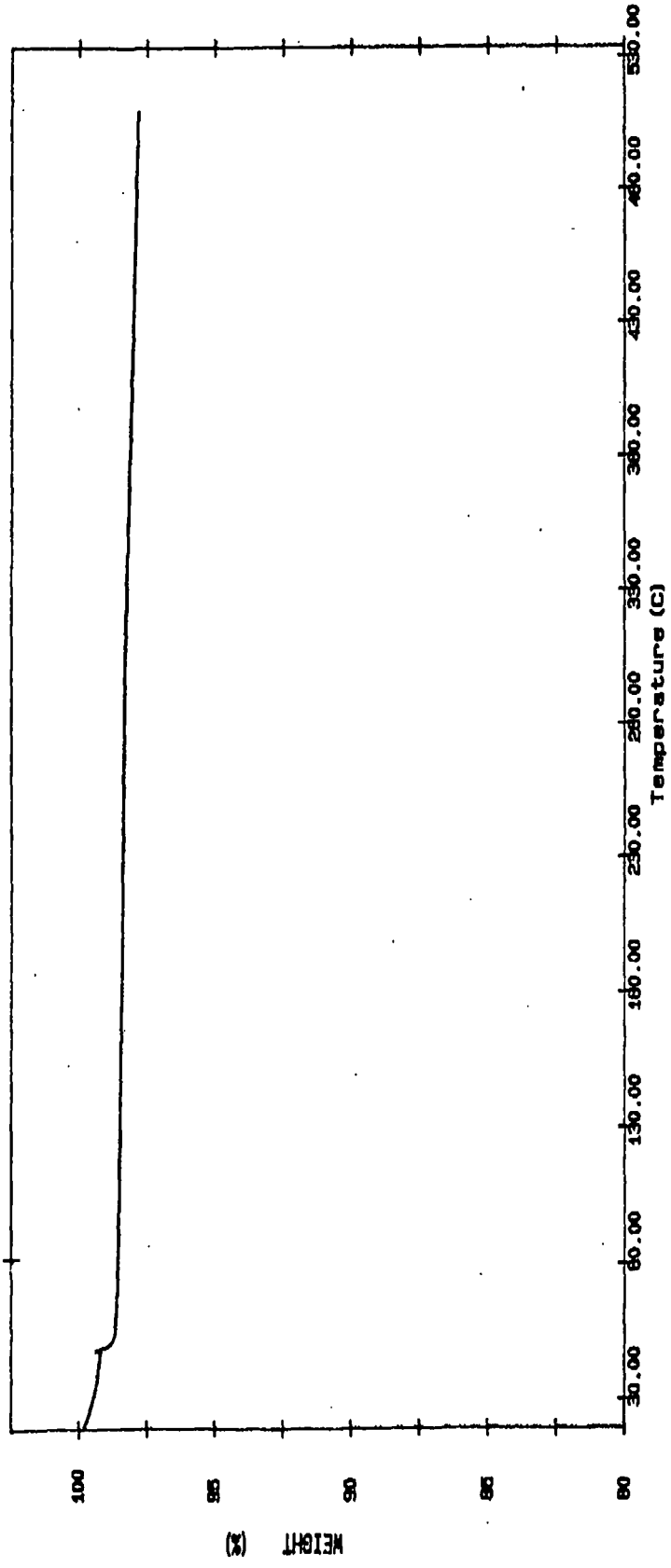
b. Zeolite A


100
87.5
75
62.5
50
WEIGHT (%)
10.00
50.00
90.00
130.00
170.00
210.00
250.00
290.00
330.00
370.00
410.00
Temperature (C)

c. Zeolite Y


100
87.5
75
62.5
50
WEIGHT (%)
10.00
50.00
90.00
130.00
170.00
210.00
250.00
290.00
330.00
370.00
410.00
Temperature (C)

d. Berengite


100
95
90
85
80
WEIGHT (%)
30.00
80.00
130.00
180.00
230.00
280.00
330.00
380.00
430.00
480.00
530.00
Temperature (C)

zeolite powders produces a scattering pattern from the regular arrays of atoms (ions) within the structure. It reflects the framework and nonframework symmetry of the constituents of each zeolite.

The dry zeolite samples were analysed at room temperature using Cu-Kα radiation on a PW 1720 X Kristalloflex difractometer. The powder pattern for zeolites P, 4A and Y coincide with those reported by Breck (1974) (Table 3.3.). The XRD pattern for berengite is also indicated in Table 3.3., although no pattern has been reported for this compound.

3.3.2.c. SEM

For electron microscopy examination, a few milligrams of sedimentated zeolite was dusted on a small metal target coated with an adhesive. The particles of zeolite were then coated with heavy and structureless platinum/gold which evaporates much more easily than pure platinum. The vacuum evaporation was carried out by a tungsten filament. The evaporation time was 1-2 sec. and the vacuum in this case was 6.5×10^{-3} Nm^{-2}. The sample holder was mounted at a 45° angle to the electron beam in the electron microscope and a series of photographs were taken. SEM photographs were recorded with an accelerating voltage of 25 kV on a Jeol JSM 840 scanning electron microscope. These are presented in Plate 3.1. (a, b, c, d).

3.3.3. Preparation of maximally exchanged zeolites

The zeolites were first exchanged several times with a solution of $NaNO_3$ (1 mol/dm^3) in order to obtain as far as possible the homoionic form of the zeolite. Approximately 20 g of each zeolite were equilibrated with $NaNO_3$ (300 ml, 1M). The solutions were shaken at room temperature for two hours. The zeolites were filtered then washed with a small amount of deionised water and dried at 70°C. The procedure was repeated three times. Finally and before use, the zeolites were equilibrated over saturated aqueous Na_2SO_4 in a dessicator at room temperature for two months.

3.3.4. Determination of Binary Ion-Exchange Isotherms.

Binary exchange isotherms were determined for the homoionic sodium forms of three synthetic zeolites and also for berengite with the metal ions, Cu^{2+}, Cd^{2+}, Zn^{2+} and Pb^{2+}.

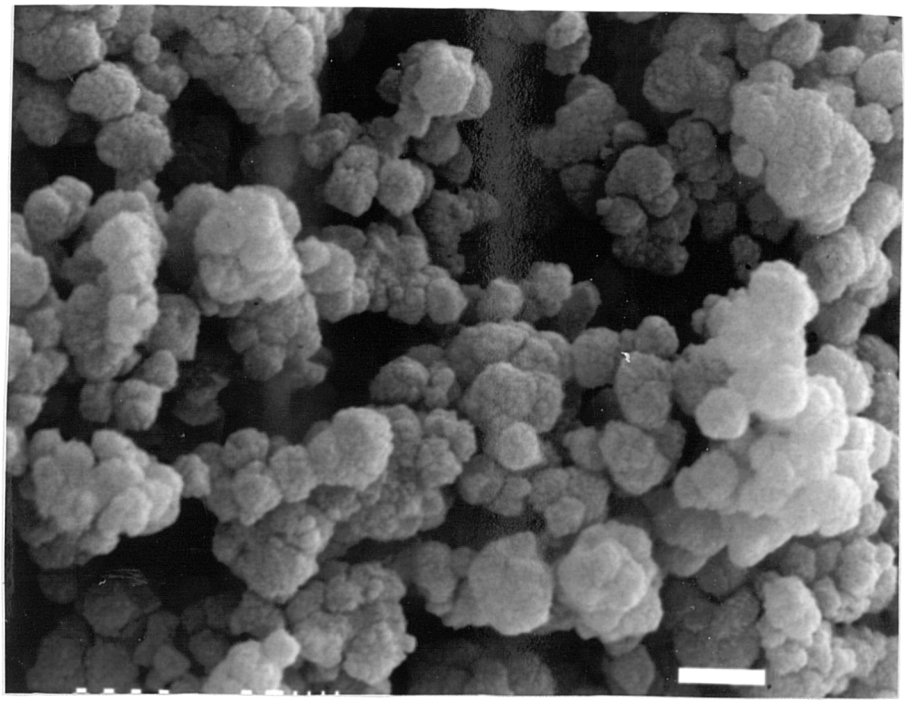
Table 3.3. XRD for Zeolites.

Zeolite P				Zeolite 4A				Zeolite Y				Berengite *	
This study		Breck, 1974		This study		Breck, 1974		This study		Breck, 1974		This study	
d(Å)	I	d(Å)	I	d(Å)	I	d(Å)	I	d(Å)	I	d(Å)	I	d(Å)	I
7.08	87.9	7.10	55	12.40	76.5	12.29	100	14.30	100	14.29	100	3.31	100
5.00	32.1	5.01	35	8.75	62.0	8.71	69	8.75	25.8	8.75	9	4.20	18.9
4.11	55.6	4.10	55	7.13	41.4	7.11	35	7.46	17.9	7.46	24	4.43	17.7
3.17	78.8	3.16	100	5.52	32.8	5.51	25	5.67	59.0	5.68	44	3.46	9.5
2.71	72.2	2.67	55	5.04	2.1	5.03	2	4.76	23.5	4.76	23	9.90	7.3

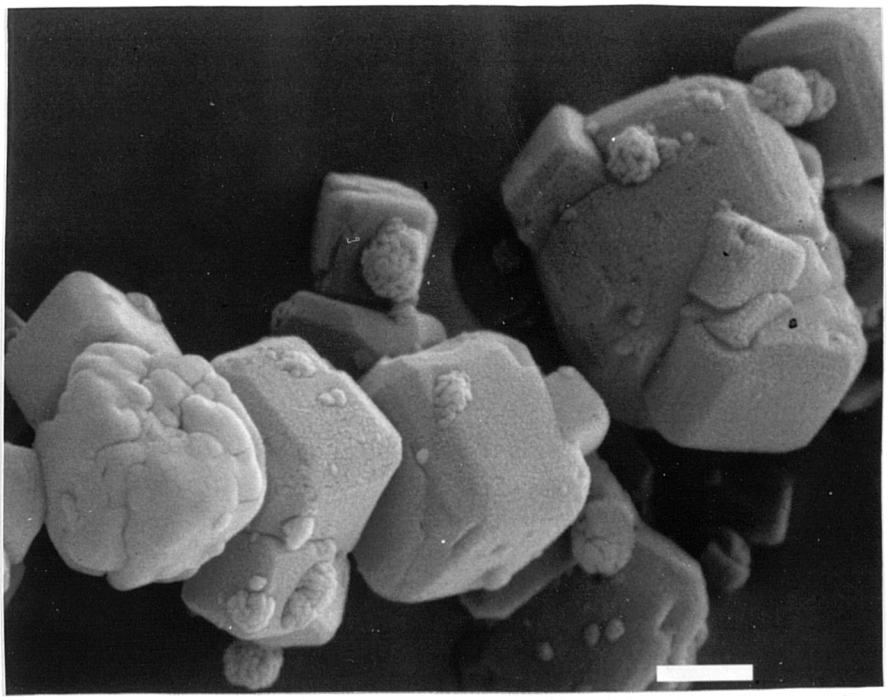
* No data available for comparison.

Plate 3.1. SEM for zeolite samples.

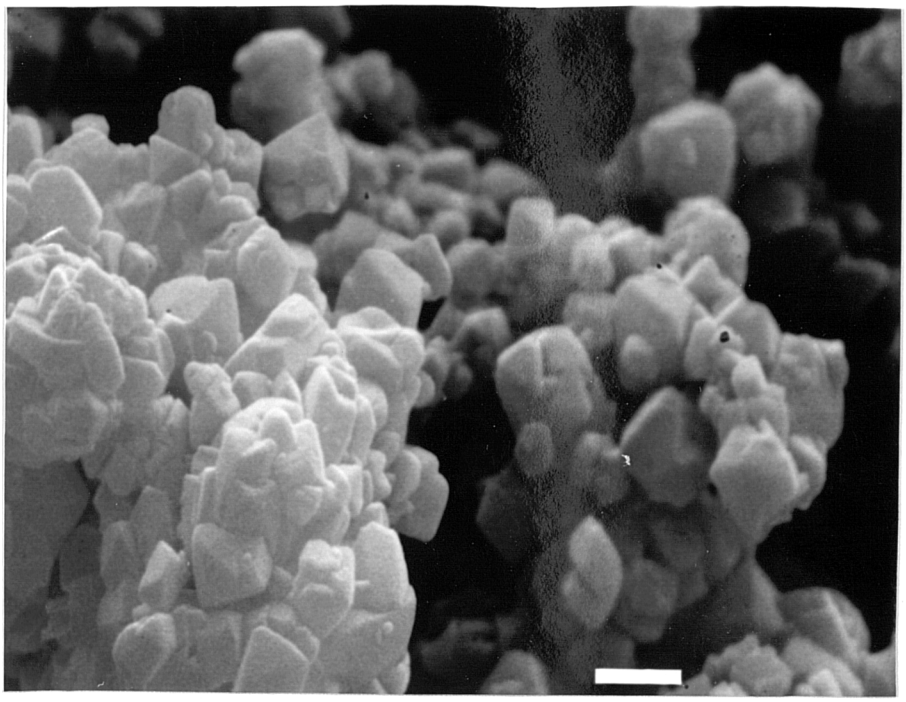
a. Scanning electron micrograph of zeolite P, gold-sputter coated. Taken using 25 kV; bar = 1 μ.



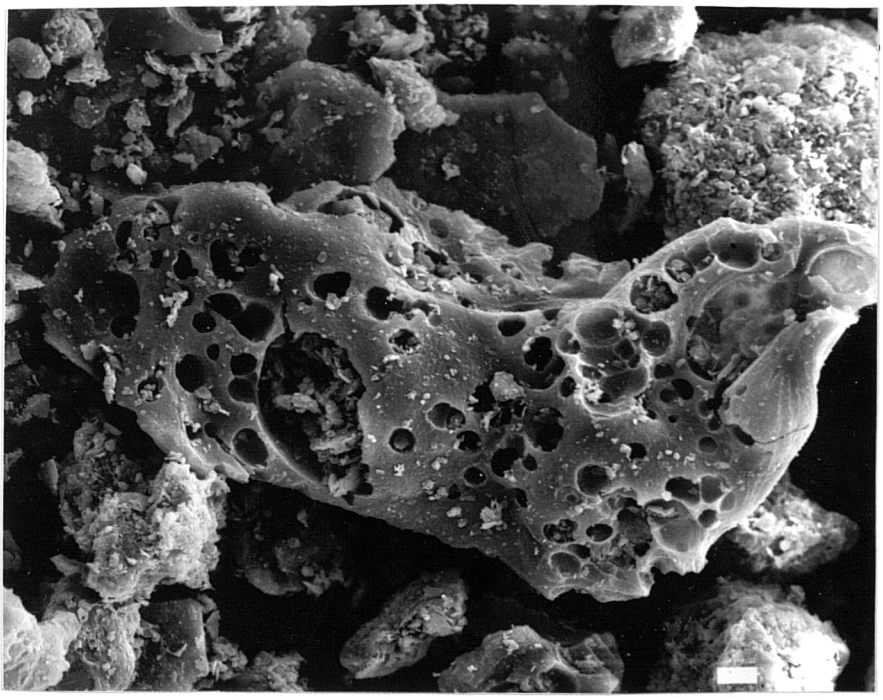
b. Scanning electron micrograph of zeolite 4A, gold-sputter coated. Taken using 25 kV; bar = 1 μ.



c. Scanning electron micrograph of zeolite Y, gold-sputter coated. Taken using 25 kV; bar = 1 μ.



d. Scanning electron micrograph of Berengite, gold-sputter coated. Taken using 25 kV; bar = 10 μ.



The isotherms were obtained by equilibrating accurately weighed portions of the zeolite in a series of solutions containing the two competing ions in different concentrations, at a constant solution normality of 0.1 mol equiv.dm^{-3}. Isotherm points were obtained by equilibrating accurately weighed samples of each zeolite (0.400 g) with 50 cm^3 portions of solution containing the appropriate quantities of copper, cadmium, zinc and lead nitrate and sodium nitrate as the counter ion.

The equilibration experiments were carried out in sealed polyethylene bottles, which were placed in a thermostatted water bath at 298.2° K (± 0.1) for 30 days with constant agitation, to ensure equilibrium had been established. Preliminary investigations indicated that equilibrium was established well before this time. The pH of all solutions was measured with a Radiometer PHM85 precision pH meter fitted with a Radiometer GK2401C combination glass electrode and adjusted to pH 5 by adding NaOH (0.1M) or HNO_3 (0.1M) at the start of the experiment to avoid hydroxide precipitation.

After equilibration the mixtures were centrifuged for 10 mins. at 4000 rpm, filtered through GF/C glass fibre filter paper and analysed immediately for metal content by AAS using a Perkin Elmer SP9 Atomic Absorbtion Spectrophotometer. Standard copper, cadmium, zinc and lead solutions were prepared in dilute nitric acid (c.a. 20%).

3.4. Results and Discussion

Binary ion-exchange isotherms have been derived for the three zeolites P, 4A and Y and also for berengite, studied under standard conditions, with aqueous solutions of copper, cadmium, zinc and lead. The degree of ion-exchange was calculated from a knowledge of the composition of the initial and final solutions, volume of solution and mass of zeolite used. The ion-exchange isotherms presented in Figs. 3.4.-3.8. were derived by calculating after equilibration, the equivalent fraction of exchanging ion in solution (As) and plotted against the equivalent fraction of exchanging ion in the solid zeolite crystal (Ac) (Tables 3.4.-3.8.). All values are the means of three replicate determinations.

Calculations were based on the maximum exchange capacity of each zeolite (Table 3.9.), although under the conditions studied, complete ion exchange was not observed in any case.

All three zeolites studied exhibit a preference for the divalent exchanging cation over sodium. The selectivity patterns observed are not equal, but dependant on the size and nature of the exchanging cation in aqueous solution.

Fig. 3.4. Ion-exchange isotherms for zinc (n=3).

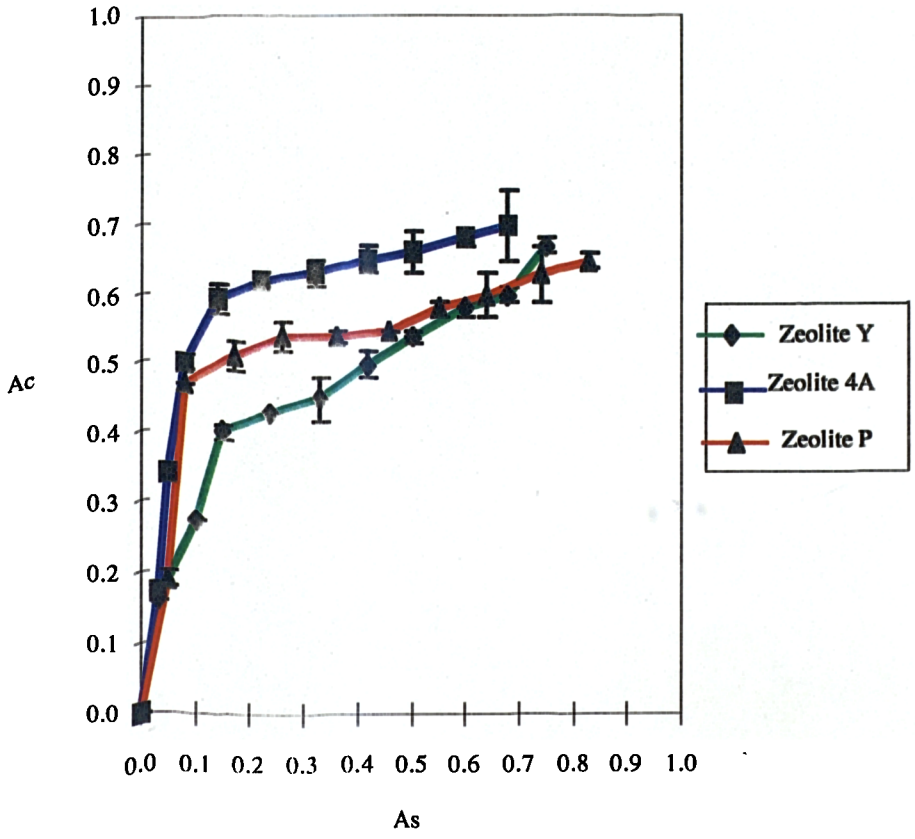


Table 3.4. Equivalent fractions for zinc ion-exchange isotherms.

(A_S = equivalent fraction of exchanging ion in solution; A_C=equivalent fraction of exchanging ion in the crystal).

	Zeo P		Zeo 4A		Zeo Y	
Sample	As	Ac	As	Ac	As	Ac
1	0.75 (±0.01)	0.67 (±0.00)	0.68 (±0.05)	0.70 (±0.03)	0.83 (±0.01)	0.65 (±0.00)
2	0.68 (±0.04)	0.60 (±0.01)	0.60 (±0.01)	0.68 (±0.01)	0.74 (±0.01)	0.63 (±0.01)
3	0.60 (±0.03)	0.58 (±0.03)	0.50 (±0.03)	0.66 (±0.01)	0.64 (±0.01)	0.60 (±0.05)
4	0.50 (±0.01)	0.54 (±0.01)	0.42 (±0.02)	0.65 (±0.03)	0.55 (±0.01)	0.58 (±0.02)
5	0.42 (±0.00)	0.50 (±0.00)	0.32 (±0.02)	0.63 (±0.02)	0.46 (±0.02)	0.55 (±0.03)
6	0.30 (±0.01)	0.45 (±0.01)	0.22 (±0.01)	0.62 (±0.00)	0.36 (±0.03)	0.54 (±0.04)
7	0.24 (±0.02)	0.43 (±0.01)	0.14 (±0.02)	0.50 (±0.04)	0.26 (±0.00)	0.54 (±0.02)
8	0.15 (±0.02)	0.40 (±0.00)	0.08 (±0.01)	0.50 (±0.00)	0.10 (±0.01)	0.51 (±0.00)
9	0.10 (±0.00)	0.27 (±0.01)	0.05 (±0.00)	0.34 (±0.01)	0.08 (±0.00)	0.47 (±0.05)
10	0.03 (±0.01)	0.16 (±0.03)	0.00 (±0.00)	0.17 (±0.02)	0.05 (±0.00)	0.19 (±0.03)
11	0.00 (±0.00)	0.00 (±0.00)	0.00 (±0.00)	0.00 (±0.00)	0.00 (±0.00)	0.00 (±0.00)

Fig. 3.5. Ion-exchange isotherms for cadmium (n=3).

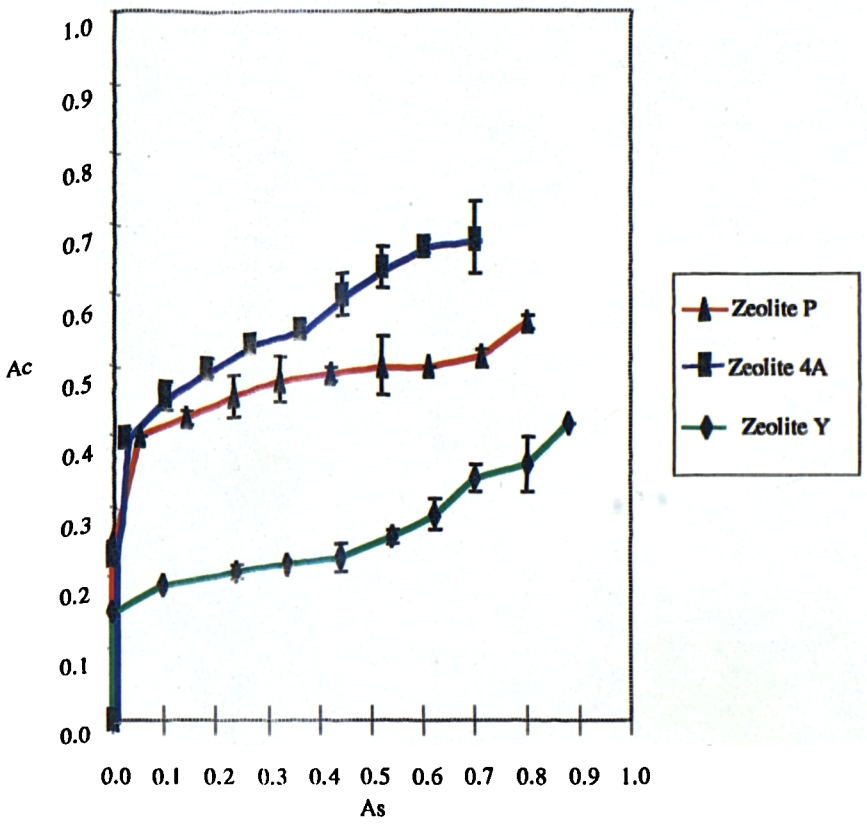


Table 3.5. Equivalent fractions for cadmium ion-exchange isotherms. (for legend see Table 3.4.).

	Zeo P		Zeo 4A		Zeo Y	
Sample	As	Ac	As	Ac	As	Ac
1	0.80 (±0.01)	0.56 (±0.03)	0.70 (±0.05)	0.68 (±0.02)	0.88 (±0.00)	0.42 (±0.01)
2	0.71 (±0.01)	0.51 (±0.01)	0.60 (±0.01)	0.67 (±0.02)	0.80 (±0.04)	0.36 (±0.01)
3	0.61 (±0.00)	0.50 (±0.03)	0.52 (±0.03)	0.64 (±0.04)	0.70 (±0.02)	0.34 (±0.01)
4	0.52 (±0.04)	0.50 (±0.01)	0.44 (±0.03)	0.60 (±0.03)	0.62 (±0.02)	0.29 (±0.03)
5	0.42 (±0.01)	0.49 (±0.00)	0.36 (±0.01)	0.55 (±0.05)	0.54 (±0.01)	0.26 (±0.04)
6	0.32 (±0.03)	0.48 (±0.04)	0.26 (±0.00)	0.53 (±0.00)	0.44 (±0.02)	0.23 (±0.04)
7	0.23 (±0.03)	0.46 (±0.03)	0.18 (±0.01)	0.50 (±0.01)	0.34 (±0.00)	0.22 (±0.00)
8	0.14 (±0.01)	0.43 (±0.00)	0.10 (±0.02)	0.46 (±0.00)	0.24 (±0.01)	0.21 (±0.04)
9	0.05 (±0.00)	0.40 (±0.03)	0.02 (±0.01)	0.40 (±0.04)	0.10 (±0.00)	0.19 (±0.02)
10	0.00(±0.00)	0.25 (±0.02)	0 90(±0.00)	0.23 (±0.00)	0.00 (±0.00)	0.15 (±0.03)
11	0.00 (±0.00)	0.00 (±0.00)	0.00 (±0.00)	0.00 (±0.00)	0.00 (±0.00)	0.00 (±0.00)

Fig. 3.6. Ion-exchange isotherms for copper (n=3).

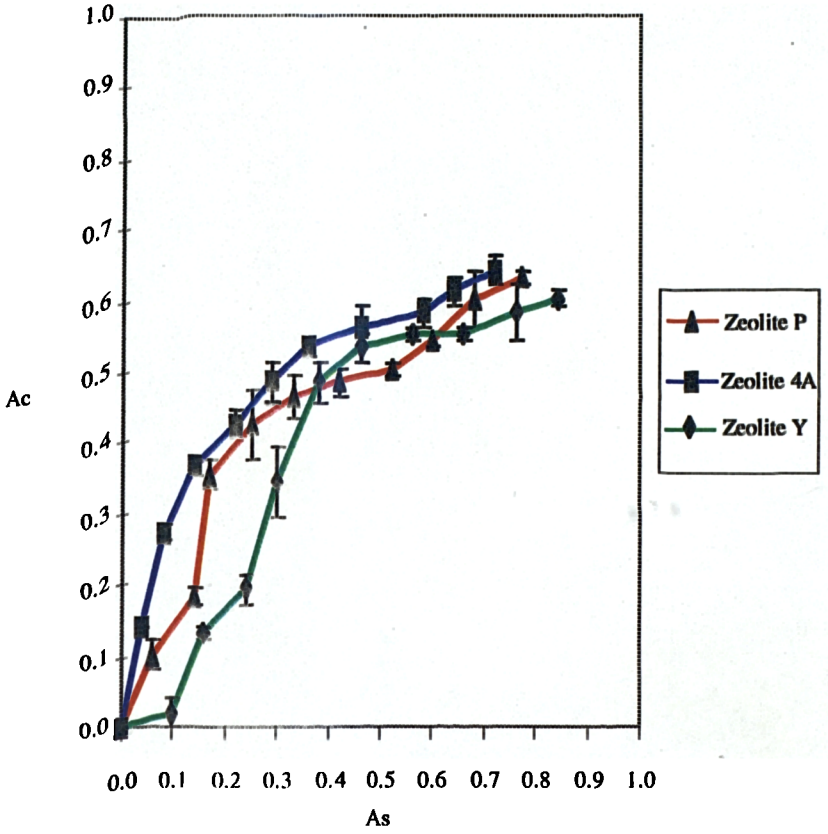


Table 3.6. Equivalent fractions for copper ion-exchange isotherms. (for legend see Table 3.4.).

	Zeo P		Zeo 4A		Zeo Y	
Sample	As	Ac	As	Ac	As	Ac
1	0.77 (±0.01)	0.63 (±0.02)	0.72 (±0.02)	0.64 (±0.04)	0.84 (±0.02)	0.60 (±0.00)
2	0.68 (±0.04)	0.60 (±0.03)	0.64 (±0.02)	0.61 (±0.02)	0.76 (±0.01)	0.58 (±0.01)
3	0.60 (±0.01)	0.54 (±0.00)	0.58 (±0.02)	0.58 (±0.01)	0.66 (±0.00)	0.55 (±0.01)
4	0.52 (±0.01)	0.50 (±0.01)	0.46 (±0.03)	0.56 (±0.00)	0.56 (±0.03)	0.55 (±0.01)
5	0.42 (±0.02)	0.48 (±0.00)	0.36 (±0.01)	0.53 (±0.01)	0.46 (±0.03)	0.53 (±0.01)
6	0.33 (±0.03)	0.46 (±0.01)	0.29 (±0.03)	0.48 (±0.00)	0.38 (±0.01)	0.48 (±0.04)
7	0.25 (±0.05)	0.42 (±0.03)	0.22 (±0.02)	0.42 (±0.03)	0.30 (±0.00)	0.34 (±0.01)
8	0.17 (±0.02)	0.35 (±0.01)	0.14 (±0.01)	0.36 (±0.00)	0.24 (±0.03)	0.19 (±0.03)
9	0.14 (±0.01)	0.18 (±0.02)	0.08 (±0.01)	0.27 (±0.00)	0.16 (±0.00)	0.13 (±0.00)
10	0.06 (±0.02)	0.10 (±0.01)	0.04 (±0.00)	0.14 (±0.01)	0.10 (±0.00)	0.02 (±0.01)
11	0.00 (±0.00)	0.00 (±0.00)	0.00(±0.00)	0.00 (±0.00)	0.00 (±0.00)	0.00 (±0.00)

Fig. 3.7. Ion-exchange isotherms for lead (n=3).

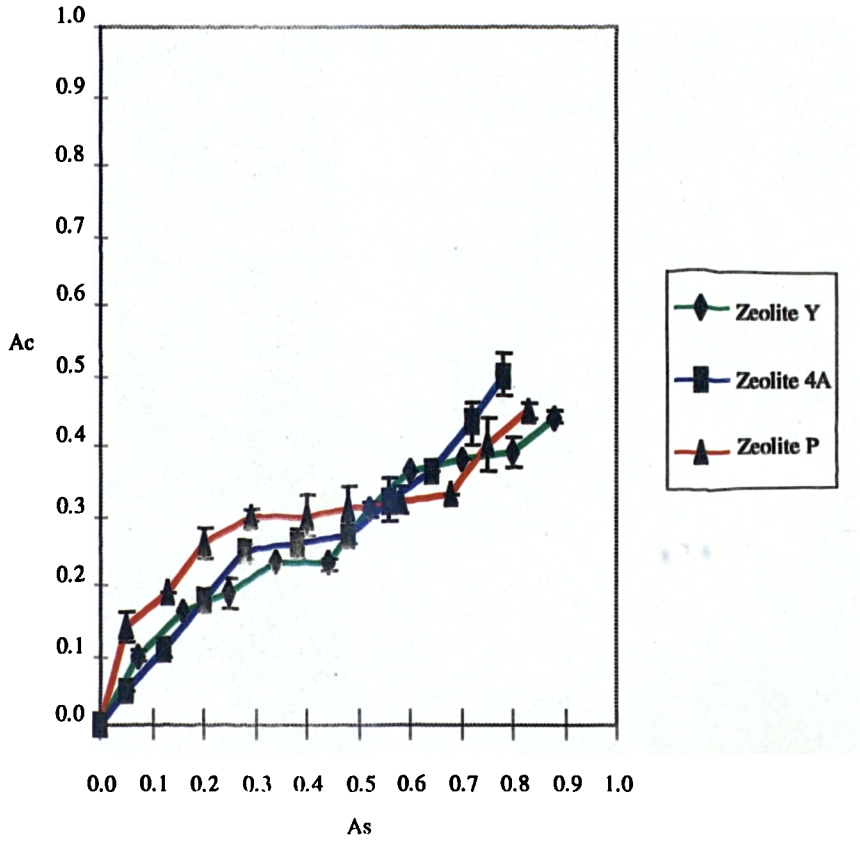


Table 3.7. Equivalent fractions for lead ion-exchange isotherms. (for legend see Table 3.4.).

	Zeo P		Zeo 4A		Zeo Y	
Sample	As	Ac	As	Ac	As	Ac
1	0.83(±0.01)	0.45 (±0.02)	0.78 (±0.03)	0.50 (±0.00)	0.88 (±0.01)	0.44 (±0.00)
2	0.75 (±0.04)	0.40 (±0.03)	0.72 (±0.03)	0.43 (±0.01)	0.80 (±0.02)	0.39 (±0.01)
3	0.68 (±0.00)	0.33 (±0.03)	0.64 (±0.00)	0.36 (±0.03)	0.70 (±0.00)	0.38 (±0.01)
4	0.58 (±0.02)	0.32 (±0.01)	0.56 (±0.03)	0.32 (±0.02)	0.60 (±0.00)	0.36 (±0.00)
5	0.48 (±0.03)	0.31 (±0.00)	0.48 (±0.01)	0.27 (±0.01)	0.52(±0.01)	0.31 (±0.02)
6	0.40 (±0.03)	0.30 (±0.02)	0.38 (±0.02)	0.26 (±0.00)	0.44 (±0.01)	0.23 (±0.04)
7	0.29 (±0.01)	0.30 (±0.03)	0.28 (±0.01)	0.25 (±0.02)	0.34 (±0.00)	0.23 (±0.03)
8	0.20 (±0.02)	0.26 (±0.01)	0.20 (±0.00)	0.18 (±0.00)	0.25(±0.02)	0.19 (±0.00)
9	0.13 (±0.00)	0.19 (±0.03)	0.12 (±0.01)	0.11 (±0.01)	0.16 (±0.00)	0.16 (±0.00)
10	0.05 (±0.02)	0.14 (±0.00)	0.05 (±0.00)	0.05 (±0.02)	0.07(±0.01)	0.10 (±0.03)
11	0.00 (±0.00)	0.00 (±0.00)	0.00 (±0.00)	0.00 (±0.00)	0.00 (±0.00)	0.00 (±0.00)

Fig. 3.8. Ion-exchange isotherms for berengite (n=3).

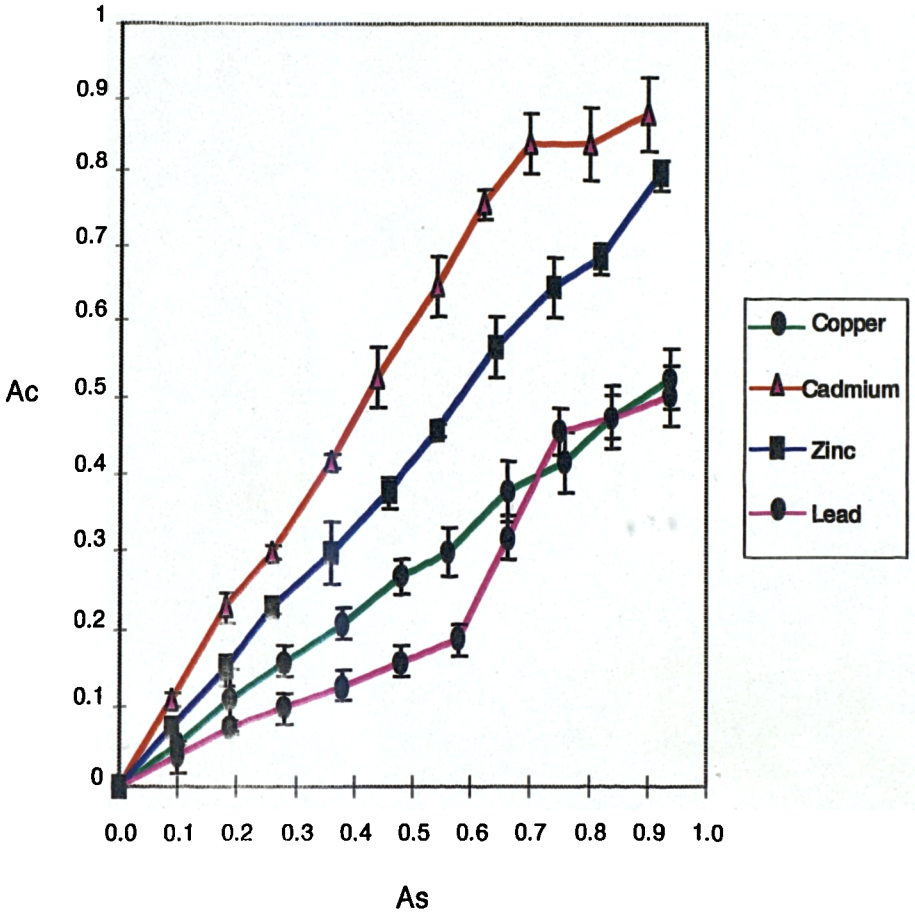


Table 3.8. Equivalent fractions for berengite ion-exchange isotherms (n=3).

Sample	Copper		Cadmium		Zinc		Lead	
	As	Ac	As	Ac	As	Ac	As	Ac
1	0.94 (±0.04)	0.53 (±0.02)	0.90 (±0.05)	0.88 (±0.02)	0.92 (±0.02)	0.80 (±0.02)	0.94 (±0.04)	0.51 (±0.03)
2	0.84 (±0.03)	0.48 (±0.02)	0.80 (±0.05)	0.84 (±0.02)	0.82 (±0.02)	0.69 (±0.01)	0.84 (±0.04)	0.48 (±0.02)
3	0.76 (±0.04)	0.42 (±0.03)	0.70 (±0.04)	0.84 (±0.04)	0.74 (±0.04)	0.65 (±0.03)	0.75 (±0.03)	0.46 (±0.02)
4	0.66 (±0.04)	0.38 (±0.02)	0.62 (±0.02)	0.76 (±0.06)	0.64 (±0.04)	0.57 (±0.03)	0.66 (±0.03)	0.32 (±0.02)
5	0.56 (±0.03)	0.30 (±0.00)	0.54 (±0.04)	0.65 (±0.03)	0.54 (±0.01)	0.46 (±0.02)	0.58 (±0.02)	0.19 (±0.01)
6	0.48 (±0.02)	0.27 (±0.03)	0.44 (±0.04)	0.53 (±0.03)	0.46 (±0.02)	0.38 (±0.02)	0.48 (±0.02)	0.16 (±0.01)
7	0.38 (±0.02)	0.21 (±0.03)	0.36 (±0.01)	0.42 (±0.02)	0.36 (±0.04)	0.30 (±0.00)	0.38 (±0.02)	0.13 (±0.03)
8	0.28 (±0.02)	0.16 (±0.02)	0.26 (±0.01)	0.30 (±0.00)	0.26 (±0.01)	0.23 (±0.03)	0.28 (±0.02)	0.10 (±0.00)
9	0.19 (±0.04)	0.11 (±0.01)	0.18 (±0.02)	0.23 (±0.03)	0.18 (±0.02)	0.15 (±0.02)	0.19 (±0.01)	0.076 (±0.0)
10	0.10 (±0.00)	0.053 (±0.0)	0.09 (±0.01)	0.11 (±0.01)	0.09 (±0.01)	0.076 (±0.0)	0.10 (±0.02)	0.038 (±0.0)
11	0.00 (±0.00)	0.00 (±0.00)	0.00 (±0.00)	0.00 (±0.00)	0.00 (±0.00)	0.00 (±0.00)	0.00 (±0.00)	0.00 (±0.00)

Table 3.9. General characteristics of zeolites.

Zeolite	Na:Si:Al Ratio	Maximum Exchange Capacity (moles/g) based on anhydrous form
P	1:1:1	2.9×10^{-3}
4A	1:1:1	2.9×10^{-3}
Y	1:2.6:1	1.54×10^{-3}
Berengite	1:2.8:35.5	0.23×10^{-3}

Each zeolite provides a different pattern of ion-exchange selectivity.

The characteristic features of the binary isotherms for all three zeolites with zinc and cadmium are very similar and exhibit the typical exchange behaviour of a simple divalent cation (Fig. 3.4. and 3.5.). At low metal concentrations, the zeolites show a high selectivity for the divalent metal over sodium, and will readily exchange sodium in the crystal for the divalent cation up to an average of 0.5 Ac (50% of the theoretical exchange capacity). Zinc and cadmium are both group 2B elements and therefore the behaviour of zinc in a zeolitic environment is expected to resemble that of cadmium.

Although copper and zinc have Pauling radii with nearly identical values (Cu = 0.72 Å and Zn = 0.74 Å), which are small enough to suggest free diffusion into the small zeolite cavities, the ion-exchange isotherms for these two metals are different (see Fig. 3.6. and 3.4.).

Copper and lead replacement is less efficient than that observed both for cadmium and zinc (Fig. 3.6. and 3.7.). Copper and lead tend to form polymeric species in neutral and slightly acidic aqueous solution (Burges, 1978), and their effective size prevents efficient exchange through the zeolite channels. The values of hydrolysis constants (pK1) for Pb^{2+} (7.8-7.9) and Cu^{2+} (7.3-8.0) are smaller than those for Zn^{2+} (9.0-9.4) and Cd^{2+} (7.6-11.6); therefore, lead and copper are more easily hydrolysed than zinc and cadmium (Elfattah and Wada, 1981). Copper solutions at different pH's can readily precipitate basic salts within the zeolite matrix to differing degrees (Ione *et al.*, 1974). Dyer and Barri (1977) have shown that hydroxy-copper species do precipitate from copper nitrate solutions on the external crystalline surfaces. To prevent hydroxy species forming, all exchanging solutions were maintained at pH 5.00.

The equivalent fraction of copper and lead in all three zeolites increases to approximately 0.5 Ac as the concentration of exchanging metal in solution increases (up to 0.7 equivalent fraction). This is in agreement with previous observations, that complete exchange of the sodium ions in the zeolite structure is rarely achieved (Jama and Yucel, 1990).

The difference in exchange between copper and lead compared to cadmium and zinc can also be related to the substantially greater water structuring properties of copper and lead compared to the other two ions, hindering diffusion to the exchange sites in the smaller cages in the zeolite (Barrer and Townsend, 1975).

All the isotherms demonstrate limited exchange and a strong preference for the divalent cation over sodium is shown in all cases by all three zeolites. The readily replaceable sodium ions originate from the α-supercage zeolite framework, whereas the sodium ions in the β-, or sodalite structure are less easily replaced, and complete exchange of sodium is highly unlikely.

The sigmoidal shape of the isotherm is due to the heterogeneous character of the ion-exchange sites (heterogeneous in the sense that cations are involved in different interactions with the lattice oxygen atoms and water molecules in the different cages). Even in the large cages the cations can occupy crystallographically different sites (Sherry, 1968). The lack of correspondence between the models of ion-exchange and the selectivity of the zeolites for given cations is often attributed to differences in the polarizability of the exchanging cations. Thus, the sigmoidal shape of the isotherms can be interpreted in terms of a pronounced selectivity of the divalent cations for the sites in the supercages, and a preference of sodium for the sites in the small cages.

Implicit in these views is that, in cases of complete exchange, the divalent ions predominantly occupy positions in the large cages in the initial phases of the exchange reaction, the sites in the small cages being taken in the very last stages of the exchange (Maes and Cremers, 1974).

Zeolite A displays a double ion-sieve action. Only small cations can penetrate the single 6-rings into the β-cages. Large ions such as organic cations cannot penetrate the 8-rings into the α-cages. Zeolite A has a high aluminium content and also a large void volume and the order of selectivity for divalent ions is: $Mg^{2+} < Hg^{2+} < Cd^{2+} < Ni^{2+} < Co^{2+} < Ca^{2+} < Ba^{2+} < Sr^{2+} < Zn^{2+}$. In synthetic zeolite 4A, the maximum exchange levels decrease from zinc

to cadmium and nickel (Maes and Cremers, 1974). In this study, the same order of preference for zinc and cadmium was observed.

Zinc ion-exchange and the factors determining the degree of exchange in zeolite A has been extensively investigated (Gal *et al.*, 1971; Coughlan and Caroll, 1976; Takaishi *et al.*, 1975; Lutz *et al.*, 1990). The maximum degree of exchange was 70% which was in agreement for the selectivity of divalent ions by zeolite A (Breck *et al.*, 1956).

The order of decreasing selectivity for the transition metals in zeolite Y is: $Ni^{2+} > Cu^{2+} > Co^{2+} > Zn^{2+} > Mn^{2+} > Cd^{2+}$ (Coughlan and Carroll, 1976). In zeolites X and Y, the copper ions are partially situated in the sodalite units (Maxwell and de Boer, 1975), access to which is limited by six-oxygen windows of free diameter ~0.22 nm (Breck, 1974), a much smaller diameter than that of the channels limiting access to the side pockets in mordenite, into which hydrated copper ions did not exchange (Fletcher and Townsend, 1980).

As the ionic radii of the transition metal ions are very much smaller than the diameters of the 6-rings governing access to the sodalite cages, the incomplete exchange of sodium by copper in zeolite Y at 25°C implies that this ion exists as distinct aqua-complex in the supercages of zeolite Y (Lai and Rees, 1975). Copper ions readily replace sodium ions in the supercages, where the selectivity is $Cu^{2+} > Na^{+}$ in the hydrated zeolite Y (Herman and Bulko, 1980).

In the case of lead, the introduced lead ions occupy the strongly coordinated positions within the β-cages and those of weekly coordinated sites in the α-cages. The strongly coordinated lead ions are situated near the double 4-rings where they disturb the symmetry of the framework as hydrated species (Steinike *et al.*, 1995).

In zeolite Y, ion exchange at 25°C is essentially restricted to the large cages, as inferred from the maximum exchange levels. A 75-80% zinc exchange seems to be the critical limit for framework stability of zeolite Y since greater than 80% zinc-exchanged Y zeolites have been reported to lead to framework collapse (Boddenberg and Seidel, 1994). In exhaustive ion-exchange experiments, Dyer and Townsend (1973) and Wolf *et al.*(1973) did observe complete exchange of zinc for sodium in zeolite Y. The selectivity of the ion exchangers for the cations Pb^{2+} and Zn^{2+} increases correspondingly from zeolite NaY to zeolite NaX, i.e., with strengthening of the electric field of the zeolite (Bobonich, 1991).

Zeolites 4A and P show similar exchange characteristics with heavy metals (Table 3.10.), whereas the amount exchanged by zeolite Y is much less, which is in agreement with the structural formulae.

Table 3.10. Efficiency of zeolites for ion-exchange.

	Exchange Capacity (mg/g)				Percentage Exchanged (%)			
Metal	Zeo P	Zeo 4A	Zeo Y	Berengite	Zeo P	Zeo 4A	Zeo Y	Berengite
Copper	100	112.4	62.4	22.3	63	64	59	53
Cadmium	140	202	95	65	55	68	42	88
Zinc	102.4	132.5	67.5	32.5	67	70	65	80
Lead	220	282	207.5	70	45	50	44	52

All four ion-exchange isotherms for berengite (Fig. 3.8.) correspond to type d isotherm (Fig. 3.1.2.d.), showing that overall berengite has no preference for either exchanging ion or counterion over the whole isotherm. The copper, cadmium, zinc and lead isotherms for berengite are straight lines and do not have the characteristical sigmoidal shape of the isotherms for the other three zeolites. This indicates a lack of selectivity for the divalent cations, and a surface adsorption phenomenon as a main sorption mechanism.

Berengite has a high pH value of 12.7 (Wessolek and Fahrenhorst, 1994), which is explained by the presence of calcium- and magnesium oxides which form hydroxides in contact with water. Both hydroxides are responsible for the high buffering capacity.

Studies by Wessolek and Fahrenhorst (1994) revealed the sigmoidal shape of the isotherms for zinc and cadmium, but only at 8% concentration of berengite. Also, these studies did not take into account the other two metals that were measured in this study, namely copper and lead. 0.8% berengite was used in this study, a concentration which is ten times lower than that used by Wessolek and Fahrenhorst (1994) in order to construct ion-exchange isotherms for berengite, therefore the differences between the results.

The equivalent fraction of exchanging ion in berengite (A_c) was higher for cadmium and zinc (0.9 and 0.8 respectively) than for copper and lead (above 0.5), in this respect berengite showing somewhat a similarity with the other three zeolites (P, 4A and Y), which also showed a higher selectivity for cadmium and zinc than for copper and lead.

The maximum exchange capacity of berengite (0.23×10^{-3} moles/g) is lower than that of zeolite Y (1.54×10^{-3} moles/g), therefore the ion-exchange isotherms indicate that berengite has a much lower selectivity for heavy metals in solution, at low concentrations compared to zeolite Y.

As inferred from the isotherms, partial levels of exchange have been obtained for all zeolites. This is a phenomenon that is commonly observed in zeolite ion exchange. At a given temperature, the ingoing ion fails to exchange to 100% of the theoretical exchange capacity of the zeolite. In effect, some sites within the crystal remain inaccessible to the entering ion. The cause of this phenomenon may be due to a number of factors. The rigid, regular crystalline structure of the zeolite, entering ions which are larger than the channel size within the crystal are excluded, so the zeolite acts as a sieve.

This "ion sieving" is often observed when the ions themselves have ionic radii which should not cause their exclusion from zeolite channels. These ions are usually strongly hydrated in aqueous solution, and their exclusion is considered to be associated with this factor.

Zeolite channels may also cause ion exclusion in another manner, connected with the total void volume of the channels. Ions are partially excluded, not because they cannot pass through the channel windows, but because they fill all the intracrystalline volume before 100% exchange is achieved. This phenomenon is known as the volume-steric exclusion mechanism. Occasionally, the ion sieve and volume steric effects appear to operate together and 100% exchange cannot be achieved. A further factor which influences the maximum exchange level is the ratio of the total available cation sites to charges on the zeolite lattice and the strength of interaction between exchanging cations (Fletcher and Townsend, 1985).

With zeolite Y, interaction with the lattice was concluded to be the most important factor in exchange. In zeolite Y, due to the reduced charge on the zeolite aluminosilicate framework the interaction between the cations and the water molecules in the large supercages results in incomplete replacement.

This is similar to the case in zeolite A where the presence of water in the large cavities influences the ease with which a divalent ion removes water and enters the smaller sodalite or β-cages (Breck, 1974).

Despite the observed partial exchange levels, the three zeolites show a high preference for the heavy metal ions over sodium ions.

3.5. Conclusions

The determination of ion-exchange isotherms for the three zeolites P, 4A and Y has identified the nature of interaction between zeolites and heavy metals, the efficiencies and capacities of each zeolite for absorbing heavy metals from solution. Among the four

types of zeolites studied, zeolites P and 4A were found to have a higher exchange capacity, providing a high percent of metal immobilisation per gram of zeolite. The exchange of a divalent metal ion into the zeolite matrix required the removal of two sodium ions in order to maintain electroneutrality. Zeolite Y and berengite have a lower availability of sodium ions (lower exchange capacity) for exchange and a weak electrostatic environment inside the matrix.

The results of the ion-exchange isotherms indicate that zeolites P and 4A were more efficient in exchanging metal ions than zeolite Y, in agreement with that predicted from their Al/Si ratio and their theoretical exchange capacity. Zeolite Y was also efficient in exchanging metal ions, but a greater amount of it is required in order to obtain the same levels of exchange as zeolites P and 4A. Because zeolites P and 4A have showed considerable ion-exchange ability, they should be preferred for further experimental tests.

After analysing the ability of synthetic zeolites to exchange heavy metals in solution, the biological impact of synthetic zeolites P, 4A and Y upon the food chain was investigated step by step, starting with their effect upon the first link of the food chain, and namely, with the soil.

The three sites which were the subject of *in situ* land remediation by synthetic zeolites in this study have been selected carefully, from a wide range of polluted areas of the landscape of Merseyside and North Wales. The criteria of selection were two-fold: 1) single to multiple metal contamination from different sources or origins and 2) the compulsory demand of finding a solution for the remediation of these polluted areas, such as an improvement of the vegetation establishment or the reuse of the land in the near future.

CHAPTER 4.

HEAVY METAL CONTAMINATED SOIL STUDIES.

4.1. Definition and classification of potentially toxic metals.

Traditionally, toxic metals have been classified as elements having a density greater than 4.5 g cm^{-3} (Lapedes, 1974) or 6 g cm^{-3} (Phipps, 1981), thus giving rise to the term 'heavy' metal. Certain of these metals such as copper and zinc are essential to living organisms in minute amounts and are referred to as 'trace' elements, although they may become toxic at higher concentrations (Alloway, 1995). The term 'heavy metal' is now generally considered to be unsatisfactory because it refers only to the density of the element and is meaningless to their behaviour in biological systems (Nieboer & Richardson, 1980). The term 'potentially toxic element' (or PTE) has been suggested instead (Alloway, 1995) but 'heavy metal' is still used widely. Current classifications of potentially toxic elements are based on the type of donor atom preferences of metal ions and the stability of the metal complexes they form (Nieboer & Richardson, 1980; Phipps, 1981). This gives more meaningful information on their toxicity, by taking their availability and reactivity into consideration. This separates the ions into the following categories:

- Class A elements = show a preference for ligands containing oxygen.
- Class B elements = show a preference for ligands containing nitrogen or sulphur.
- Borderline elements = having preferences which are intermediate, lying between class A and B.

The metals investigated in the present study are copper, cadmium, zinc and lead, all of which are 'borderline' elements according to the classification system. They are commonly studied in ecotoxicology and have been chosen due to their prominent status as pollutants, the growing problem of their deposition in soils as a result of industrial activity and their availability and toxicity to plants.

Each metal has certain characteristics that influence its availability and toxicity to biological systems, such as organic affinity and behaviour in biological systems. In general, a metal which readily forms hydroxy complexes is sorbed onto soil components to the greatest extent and is less 'available' or active within a biological system (Alloway, 1995).

The degree of attenuation of a pollutant by soil depends upon the water content of the soil. Normally soil has a greater surface area at liquid-solid interfaces so that

absorption and ion-exchange processes are favored. Replacement of Si (IV) with Al (III) in the basic SiO_2 chemical unit in the crystalline lattice of some clay minerals yields sites

$$[SiO_2] + Al\ (III) \longrightarrow [AlO_2^-] + Si\ (IV)$$

with a net negative charge. Similarly, replacement of Al (III) by a divalent metal ion such as Mg (II) in the clay crystalline lattice produces a net negative charge.

Heavy-metal ions may be sorbed by the soil, held by ion-exchange processes, interact with organic matter in soil, undergo oxidation-reduction processes leading to mobilisation or immobilisation, or even be volatilized as organometallic compounds formed by methylating bacteria. A large number of factors affect heavy metal mobility and attenuation in soil. These include pH, pE, temperature, cation exchange capacity and nature of soil mineral matter. Normally, the mobility of heavy metals in soil and mineral matter is relatively low. A study of the relative mobilities in clay mineral columns (Griffin and Shimp,1978) showed that lead, zinc, cadmium and mercury were strongly attenuated by the clay, primarily by precipitation and exchange processes. Metal cations are readily held by ion-exchange processes and precipitation on soil:

$$2\ Soil\}^-H^+ + Cu^{2+} \longrightarrow (Soil\}^-)_2\ Cu^{2+} + 2H^+$$

$$Cu^{2+} + 2OH^- \longrightarrow Cu(OH)_2\ (s)$$

Two aspects of precipitation process are particularly important in determining the fate of hazardous ionic solutes in water. If precipitation occurs very rapidly and with a high degree of supersaturation, the solid tends to form a large number of small colloidal particles that may persist in the colloidal state for a long time. In this form, hazardous substances are much more mobile and accesible to organisms than as precipitates. A second important consideration is that many heavy metals are coprecipitated with hydrated iron (III) oxide ($Fe_2O_3.xH_2O$) or manganese (IV) oxide ($MnO_2.xH_2O$) (Manahan, 1994).

Specific adsorption increases with decreasing pK (hydrolysis) values, but in the case of lead and copper which have the same value, lead is more strongly adsorbed due to its greater ionic size. The equilibrium constants are given in Table 4.1., which also includes the characteristics of the toxic metals used and information relevant to their behaviour in biological systems (Lepp, 1981; Alloway, 1995).

Table 4.1. Information about the heavy metals analysed in this study.

Element	Natural Source	Pollution Source	Normal Levels (mgl^{-1})	pK	Predominant species in soil (pH)	Effects of metal on plants
Copper*	Occurs in pure metal state; also minerals malachite (Cu_2CO_3); chalcocite (Cu_2S).	Mining and smelting; fertilisers, sewage sludge application.	1-20	7.7 strong	$[Cu(H_2O)_6]^{2+}$ at pH < 7 $Cu(OH)^0_2$ in neutral and alkaline soils.	Stunting of growth (Foy *et al.*, 1978) & inhibition of root growth (Daniels *et al.*, 1972).
Cadmium	Black shales	Phosphatic fertilisers, zinc ores, sewage sludge and incineration of plastics.	0.1-1.0	10.1 weak	Free ion Cd^{2+} predominant but neutral $CdSO_4$ or $CdCl_2$ at pH > 6.5.	Damage to photosynthetic function (Clijsters & Van Assche, 1985).
Zinc *	Sphalerite & wurtzite (Zn,Fe)S.	Burning of coal and other fossil fuels; aerial fallout; sewage sludge, agrochemicals.	50	9.0 weak	Zn^{2+} below pH 7.7; $ZnOH^+$ up to 9.11; above this the neutral $Zn(OH)_2$ is predominant.	Photosynthetic electron transport (Baker *et al.*, 1982; vacuolation in root meristem cells (Davies *et al.*, 1991); root meristem size and root hairs (Powell *et al.*, 1988).
Lead	Black shales; galena (PbS).	Vehicle exhaust fumes; mining and smelting; agricultural materials.	17-29	7.7 strong	Calcareous soils $PbCO_3$; non-calcareous $Pb(OH)_2$, $Pb_3(PO4)_2$	Inhibition of growth and mineral nutrition (Breckle & Kahle, 1992).

* indicates that the element is plant-essential.

4.2. Soil and Site Characteristics

Laboratory experiments involving binary ion-exchange isotherms have demonstrated the ability of synthetic zeolites to undergo ion-exchange with different metals in solution (Chapter 3). The ability of synthetic zeolites to exchange cations makes them attractive alternatives for removing undesirable heavy metal ions from polluted soils. Soil samples were collected from different sites, which were polluted with different contaminants. The three sites studied represent three main sources of metal pollution: sewage sludge (Gateacre), smelting activities (Prescot) and mining (Trelogan). All three sites are contaminated with heavy metals to phytotoxic levels.

Gateacre (O.S.Grid reference SJ 878 341)

Very little is known regarding this site in terms of heavy metal pollution. The site is a field next to a sewage farm owned by North West Water plc. It has had sewage sludge applied to it from the 1960's, but this practice was ceased about 1989 due to concerns regarding metal contamination.

The field is contaminated with a number of heavy metals, including high levels of cadmium, lead, zinc, copper, nickel and mercury. The field was previously farmland, owned by Liverpool County Council until 1974, when it was sold to what is now North West Water plc. Crops farmed on the land included potatoes, barley and wheat by rotation, however the land has been under grass for twelve years, but is still cut for silage.

Prescot (O.S.Grid reference SJ 464 926)

Aerial-contaminated soil was collected from Prescot, a small town 9 km to the east of Liverpool. The main pollution input to the site is copper, and to a lesser extent cadmium and zinc from nearby metal refining industry. Industrial operations began in 1906, converting scrap copper into copper wire. This was superseded in 1932 by a copper fire refinery which produced copper and cadmium alloys and high grade copper for use as rods and anodes. Soils downwind of the plant received substantial inputs of copper and cadmium in the form of dust, particulates and aerosols. The history of this site has been reviewed by Dickinson *et al.* (1996), who showed that cessation of the activities of the refining plant may have led to a slow decrease in the levels of plant-available heavy metals.

Soil was collected from a woodland site adjacent to a churchyard which was dominated mainly by *Acer pseudoplatanus* L. (sycamore) and to a lesser extent *Aesculus hippocastaneum* (horse chestnut). Numerous studies have been undertaken in the vicinity of the smelter concerned with the evolution of tolerant grass populations (Bradshaw & McNeilly, 1981; Wu & Antonovics, 1978), movement of metals through food chains (Hunter *et al.*, 1987 ab), and more recently on the effect of emissions on sycamore trees (Dickinson *et al.*, 1992; Watmough, 1994).

Trelogan (O.S.Grid reference SJ 185 774)

The material collected from Trelogan is the spoil from an old abandoned lead and zinc mine which has not been worked since at least the turn of the century. Mining activities at Trelogan ceased around 1900, and grass species such as *Agrostis tenuis* and *Agrostis stolonifera* have invaded in sparse patches around the spoil. A few tree species on the site include sycamore (*Acer pseudoplatanus* L.), several willows (*Salix caprea* and *Salix cinerea*) and a small number of young oaks (*Quercus* sp.).

Direct revegetation of the area is not possible, mainly because of the high metal concentrations in the soil. Other limiting factors for plant growth are macronutrient deficiency, dryness and an almost complete lack of organic material.

The parent rock underlying Trelogan spoil is limestone, typically rich in calcite giving the soil a neutral pH; Smith & Bradshaw (1979) found a pH of 7.1 at this site and high calcium concentrations between 41200 and 69400 $\mu g\ g^{-1}$, with between 768-1050 $\mu g\ g^{-1}$ potassium, 125-392 $\mu g\ g^{-1}$ phosphorus and approximately 600 $\mu g\ g^{-1}$ total nitrogen. The vegetation that does grow is limited to those which can tolerate extremely alkaline and nutrient-poor conditions (Mench *et al.*, 1989).

4.2.1. Experimental (Soil Analysis)

4.2.1.1. Soil sampling

Soil for subsequent use in the pot experiments and for metal analysis was collected at the sites described above to a depth of 10 cm. Sub-samples were removed and analysed for

water- and nitric acid- extractable concentrations of copper, cadmium, zinc and lead. All samples were analysed in triplicate.

4.2.1.2. Analysis of soil characteristics.

Water-extractable and nitric acid-extractable heavy metal concentrations were determined for all three test soils. A standard potting compost (John Innes No.1) was used as a reference, uncontaminated soil.

Analysis of physical soil characteristics for Trelogan, Prescot and Gateacre soils and the reference soil were also determined, they consisted of:

- pH
- % weight loss at 105°C (moisture content)
- % weight loss on ignition (organic matter content)
- exchangeable cations (calcium, potassium and magnesium)

pH

To measure the pH of the soil, 5 g of fresh soil was mixed with 12.5 cm^3 of distilled deionised water. The soil-water mixture was stirred thoroughly using a glass rod and allowed to stand for 10 minutes before reading the pH. The pH of test soils was recorded using a Radiometer PHM85 precision pH meter fitted with a calibrated Radiometer GK2401C combination glass electrode. The mean pH of the 3 replicates was calculated for each soil.

Moisture Content

For each soil, 3 replicates of 20.00 g of fresh sample was weighed and placed in evaporating crucibles. The samples were then placed in an air-circulation oven at 105°C and dried to a constant weight, where successive weighings did not differ more than 1-2 mg. The samples were then cooled in a dessicator and weighed (Allen, 1989). Percentage moisture was then calculated using the formula:

$$\text{Moisture (\%)} = \frac{\text{loss in wt on drying (g)}}{\text{initial sample wt (g)}} \times 100$$

Organic Matter Content (Loss on ignition)

5.00 g of oven-dried soil was weighed into a dry crucible of known weight. Samples were placed in a muffle furnace and the temperature was allowed to rise to 550°C and maintained at this temperature for 3 hours before removing and cooling in a dessicator. Samples were weighed and organic matter was calculated as percentage weight loss. The loss-on-ignition (%) was calculated using the following formula:

$$\text{Loss-on-ignition (\%)} = \frac{\text{wt loss (g)}}{\text{oven-dry wt. (g)}} \times 100$$

Exchangeable cations

10.00 g of air-dried sieved soil (0.5 mm) was extracted with NH_4OAc (250 cm^3; 1M; pH 7) and filtered using Whatman N°44 filter paper. The concentration of displaced cations (principally calcium, potassium and magnesium) were determined by flame photometry and expressed as meq $100g^{-1}$.

Water-extractable metal concentration.

The fraction of the total metal content of soil that can be extracted with water may be taken as a simple representation of the concentration of metals immediately available for uptake by plants. The method described below has been shown to be successful for a variety of metal-contaminated soils, and correlates well with plant uptake of copper, nickel and zinc (Ure, 1990; Watmough, 1994). For each soil sample, 3 replicates of air dried soil (5.00 g) was shaken with distilled deionised water (25 cm^3) for 2 hours. The sample was filtered

through Whatman N°1 filter paper into 50 cm^3 volumetric flasks and diluted to volume using deionised distilled water. Metal concentration was determined immediately by atomic absorption spectroscopy.

Nitric acid-extractable metal concentration using microwave digestion.

All soil samples used were digested by microwave digestion using a MDS-81D microwave oven, Teflon PFA vessels (120 cm^3 size) with pressure relief valves and capping station (CEM Corporation). This allowed a more complete digest and reduced the risk of losing sample contents.

Triplicate soil samples were dried in an air-circulation oven to a constant weight at 105°C and ground to a fine powder using a mechanical mill. Approximately 1.0000 g of sample was added to the Teflon digestion vessels and HNO_3 (10 cm^3; conc, A.R.) added. The safety valve and caps were placed on and tightened in the capping station. After placing the samples in the turntable the exhaust fan was switched on and the rotating turntable was activated. The microwave was programmed as follows: step 1: 2 minutes 30 seconds at 100% power, step 2: 10 minutes at 80% power. The solutions were then allowed to cool for 5 minutes and then manually vented to release pressure. After cooling in a fume cupboard the solutions were filtered (Whatman N°1 filter paper) into volumetric flasks (25 cm^3) and diluted to volume using deionised, distilled water. The samples were analysed by atomic absorption spectroscopy. One nitric acid blank was included per run. All samples were analysed using a Pye Unicam SP9 Atomic Absorption Spectrophotometer at standard operating conditions, using a deuterium lamp with background correction. AAS operating conditions are presented in Table 4.2.

4.3. Results and Discussion

The physical characteristics of the soils studied are shown in Table 4.3., and metal concentrations are shown in Table 4.4. All figures are means with the standard deviation for n = 3. The normal and critical concentrations of heavy metals in polluted soils are indicated in Table 4.5.

The soils differed both in their physical characteristics (Table 4.3.) as well as in the concentrations of metals (Table 4.4.). John Innes Compost contained a greater moisture and organic matter content than all three contaminated substrates, although the organic matter

Table 4.2. Atomic absorption spectrophotometer operating conditions.

Instrumental Parameters	Copper	Cadmium	Zinc	Lead
Light source	Hollow cathode	Hollow cathode	Hollow cathode	Hollow cathode
Lamp current (mamps)	5	3	5	5
Wavelength (nm)	324.7	228.8	213.9	217.0
Slit Width (Tm)	320	320	320	320
Flame description	lean, blue	lean, blue	lean, blue	lean, blue
Sensitivity (at 0.0044 Å = 1% absorption)	0.04 Tg ml^{-1} (1Tg ml^{-1} = 0.1 Å)	0.01 Tg ml^{-1} (0.25 Tg ml^{-1} = 0.1 Å)	0.02 Tg ml^{-1} (0.5 Tg ml^{-1} = 0.1 Å)	0.1 Tg ml^{-1} (2 Tg ml^{-1} = 0.1 Å)
Optimum Working Range (ppm)	2 - 8	0.5 - 2	0.4 - 1.6	5 - 20

Source: Perkin Elmer Standard Operating Conditions Manual.

Table 4.3. Background edaphic factors in the four soils used in the experiments (Means ± standard deviation).

Soils	pH	Organic Matter (%)	Moisture Content (%)	Exchangeable Cations (meq $100g^{-1}$)	NH_4OAc-extractable Ca ($\mu g\ g^{-1}$)
Control	5.6 (±0.8)	73.8 (±2.78)	67.3 (±0.92)	290 (±17.56)	140 (±1.15)
Trelogan	7.3 (±0.22)	3.5 (±0.14)	17.8 (±2.42)	316 (±54)	6100 (±1000)
Prescot	3.4 (±0.13)	27.3 (±1.01)	47.1 (±1.16)	8.7 (±2.33)	1.3 (±1.14)
Gateacre	5.3 (±0.8)	11.7 (±0.62)	22.1 (±3.60)	140 (±9.5)	50 (±4.7)

Table 4.4. Water-extractable and nitric acid-extractable (total) metal concentrations in samples of soils used in experiments (Means ± standard deviation).

Soils	H_2O-extractable metals ($\mu g\ g^{-1}$)				HNO_3-extractable metals ($\mu g\ g^{-1}$)			
	Cu	Cd	Zn	Pb	Cu	Cd	Zn	Pb
John Innes Compost	0.11 (±0.03)	0.11 (±0.03)	0.63 (±0)	*	47.2 (±1.62)	3.43 (±0.3)	40.1 (±3.64)	20.13 (±2.61)
Trelogan	0.68 (±0.2)	5.87 (±0.28)	72.6 (±6.8)	5.21 (±0.21)	90 (±21)	80 (±18)	17700 (±6042)	10300 (±1712)
Prescot	10.7 (±0.56)	0.81 (±0.49)	2.83 (±0.3)	2.86 (±0.05)	1100 (±279)	5.4 (±2.4)	400 (±31.6)	500 (±118)
Gateacre	1.82 (±0.27)	11.21 (±0.14)	4 (±0.7)	5.18 (±0.25)	74.5 (±23)	25 (±13)	280 (±36)	575 (±38.4)

* indicates the metal concentrations were below detection limits (see Table 4.2.).

Table 4.5. Normal ranges of heavy metals concentrations in soils and plants (Alloway, 1995).

Element	Normal Range In Soil ($\mu g\ g^{-1}$)	Critical Range In Soil ($\mu g\ g^{-1}$)	Normal Range In Plants ($\mu g\ g^{-1}$)	Critical Range In Plants ($\mu g\ g^{-1}$)
Copper	2-250	60-125	5-20	20-100
Cadmium	0.01-2.0	3-8	0.1-2.4	5-30
Zinc	1-900	70-400	1-400	100-400
Lead	2-300	100-400	0.2-20	30-300

content at Prescot was still relatively high. Previous studies on soil from Prescot have shown that rates of leaf litter decomposition are slower due to the toxicity of the metals, particularly copper to the microorganisms which decompose the organic matter (Coughtrey *et al.*, 1987). Trelogan spoil has a low moisture content; the substrate is a mixture of fine sandy silt with a high clay content, suggesting that percolation of water through the spoil is less important than run-off over the surface. Gateacre has intermediate values between Trelogan and Prescot.

The concentration of exchangeable cations (an indication of CEC) of the four soils studied also differed (Table 4.3.). There are typically large differences between the CEC of mineral and organic soils; in the former CEC can range from a few to 60 meq kg^{-1} (Alloway, 1995). The cations measured to determine the concentration of total exchangeable cations were calcium, potassium and magnesium. The control soil (John Innes No.1) contained a high percentage of organic matter, which also contributes to CEC (due to high absorptive capacity above pH 5), and the concentration of exchangeable bases in the control soil is that expected for normal levels in organic soils. The concentration of exchangeable cations in Trelogan soil (316 meq 100 g^{-1}) was much higher than the control due to abundance of calcium in the substrate, with Prescot having the lowest concentration (8.7 meq 100 g^{-1}). The concentration of ammonium acetate-extractable calcium has also been included in Table 4.3. due to the ameliorating effect calcium can have on metal toxicity under normal circumstances (Wilkins, 1978). Results of soil analysis carried out in this study are generally in agreement with Smith & Bradshaw (1979).

In the soil test, all substrates were tested for metal concentration, where the analysis took only four metals into consideration, although there may conceivably have been more guest elements present in the soils, especially in Trelogan and Gateacre soils.

Trelogan soil contained much higher concentrations of cadmium, zinc and lead than those from Prescot and Gateacre, and also presented a harsher edaphic environment, which may have contributed towards the poor survival rate of vegetation in the area. The high concentration of ammonium acetate-extractable calcium is likely to be responsible for the neutral pH of the substrate which would also reduce the availability of essential metals for uptake by plants.

The total copper content of Prescot was particularly high; with 1100 $\mu g\ g^{-1}$ present, of which 10.7 $\mu g\ g^{-1}$ was water-extractable. The plant available copper concentration in Trelogan soil was 0.68 $\mu g\ g^{-1}$ and in Gateacre soil 1.82 $\mu g\ g^{-1}$ (Table 4.4.). The water-extractable concentration of cadmium in Trelogan soil was 5.87 $\mu g\ g^{-1}$; that of Prescot soil

0.81 μg g^{-1}, whereas that of Gateacre soil 1.82 μg g^{-1}. The concentration of water-extractable zinc in Trelogan soil was 72.6 μg g^{-1}, which is much higher than that of Prescot soil (2.83 μg g^{-1}) and also greater than that of Gateacre soil (4 μg g^{-1}). Concentrations of lead removed using water extraction were equal for Trelogan and Gateacre soils, lower for Prescot soil and below detection limits (approximately 1.0 μg g^{-1}) for John Innes No. 1 Compost.

Metal contamination at the sites considered in the present study occurred either as a result of aerial metal deposition, at Prescot, as a result of mining activities at Trelogan and at Gateacre, a field where sewage sludge has been applied.

Prescot, Trelogan and Gateacre were all metal contaminated sites, but differed not only in their metal concentrations, but also in vegetation cover. Trelogan soil has very high levels of metals, particularly cadmium, lead and zinc. The low organic matter of Trelogan in comparison to the other soils implies that only a minor fraction of the metals will be complexed by organic ligands. However, metal mobility will be counteracted to some extent by the pH of the soil (pH 7.3). Zinc, lead and cadmium were present in potentially phytotoxic concentrations at Trelogan (Table 4.4.), but the high soil pH may have reduced metal availability and uptake by plants. It is noteworthy that total concentrations of zinc, lead and cadmium were lower beneath an area occupied by sycamore trees than in the predominantly non-vegetated areas of Trelogan spoil. This may explain the establishment of vegetation in this area. In appearance, the absence of vegetation at Trelogan indicated the most severe phytotoxic conditions of the three sites studied, with large areas of bare spoil remaining even after several decades, apparently with little disturbance, and with no shortage of potential colonising species from nearby fields. The Gateacre site by comparison is vegetated, implying less phytotoxic conditions than found at Trelogan.

Cadmium, copper, lead and zinc are elevated above background levels at Prescot, but only copper is present at levels which could be potentially phytotoxic (Hunter *et al.*, 1987a; Turner, 1991). For Prescot soil, the mobility of metals resulting from the low pH may be compensated to some extent by the high organic matter content of 27.33%; more metal will be immobilised by metal-organic complexation.

4.4. Conclusions

The next chapter reports a sequential extraction procedure to evaluate the efficiency of synthetic zeolites: P, 4A and Y for reducing the bioavailable metal fraction in polluted soils and also to investigate the optimum working range of zeolite

concentrations for use in further experiments for assessing the feasibility of using zeolites in field trial experiments. This is the starting point to determine the direct impact of the zeolites upon polluted soils.

The effect of the synthetic zeolites: P, 4A and Y on the bioavailable fraction of metals in the contaminated soils was investigated first by using a sequential extraction procedure on the untreated and amended soils, in order to evaluate the metal redistribution in the analysed fractions after adding zeolites to the soils. Secondly, soil column experiments were carried out to measure the metal content of the leachates and therefore to estimate the long-term effect of the three synthetic zeolites upon the test soils. Laboratory investigations were followed by greenhouse trials using four test plants: ryegrass, sunflower, maize and willow grown on zeolite amended soils. Plant growth and heavy metal accumulation by the test plants were evaluated using visual examination of the plants, AAS measurements and statistical analysis of the data. A field experiment was also carried out at Prescot B.I.C.C., using zeolites P and 4A as soil amendments.

CHAPTER 5.
INVESTIGATION OF CHANGES IN METAL SPECIATION OF ZEOLITE AMENDED SOILS.

5.1. Introduction

The toxicity of metals in the natural environment depends on their chemical form. Trace metals may be distributed among many components of the soil or sediment and may be associated with them in different ways. The nature of this association has often been referred to as *speciation.* Speciation is defined as the particular physical and chemical form in which an element occurs. It is the chemical *species* or *form* that determines the mobility and bioavailability of the soil metals to other environmental compartments (such as water, plants and biota) when physicochemical conditions are favourable (Ramos *et al.*, 1994).

Metal cations in soils may be present in several different physicochemical forms: (i) as simple or complexed ions in soil solution; (ii) as easily exchangeable ions; (iii) organically bound; (iv) occluded by or coprecipitated with metal oxides, carbonates, or phosphates and other secondary minerals; or (v) as ions in crystal lattices of primary minerals (Viets, 1962; Soon & Bates, 1982). Metal cations present in soil in the first three forms are considered to be the most "available" to plants, successive forms representing decreasing degrees of availability (Soon & Bates, 1982).

The total metal content of a soil is not a reliable indicator of the metal concentrations in plants grown in that soil since only some fraction of the total metal content is available for plant uptake (Davies, 1992). Total analysis may give information concerning possible enrichment of the soil with heavy metals, but generally and for most elements, there is not sufficient criteria for estimating their biological effects. This is because it is the chemical form of a metal in the soil and sediment that determines its mobilisation capacity and behaviour in the environment (Ramos *et al.*, 1994).

A clear understanding of metal bioavailability to plants is an essential pre-requisite to reducing metal accumulation in the human food chain and preventing their potentially harmful effects on human health.

Sequential extraction or fractionation of trace metals from soils and sediments is a useful technique for determining chemical forms of metals in soil materials. Such information is valuable in predicting bioavailability, metal leaching rates, and transformations between chemical forms in agricultural and polluted soils (Salomons and Forstner, 1980). It can also

be used for monitoring a site to determine if chemical changes are occurring that may threaten the environment (Clevenger, 1990).

Selectivity of the extraction reagents toward specific geochemical phases is an important performance criterion for a sequential extraction procedure. The challenge of devising extraction schemes for transition metals is selecting reagents that are effective in selectively solubilizing a given form of the element from the soil (Salomons and Forstner, 1980).

A wide variety of extractants have been used as a means of selectively removing particular metal species from the bulk of the sample. They generally consist in solubilizing fractions which are considered as available, accessible, mobile or simply extractable. From the agricultural point of view, the extractions aim to determine amounts which are as narrowly as possible correlated with plant uptake and if possible also with crop yields.

The transfer of mineral nutrient elements from their storage form to the liquid phase is based on the following phenomena: (i) solubilization of components in the solid phase; (ii) ion-exchange (desorption); (iii) formation of soluble complexes.

Therefore it seems logic to base the extraction on the same mechanisms and to use an extracting solution responding to the following criteria: (i) be sufficient acid to solubilize the solid phase fraction which contributes to plant nutrition; (ii) contains a displacing ion which exchanges the mobile adsorbed ions; (iii) have a sufficient complexing power to form soluble organo-mineral complexes (Kiekens and Cottenie, 1982).

Generally, these schemes use a sequence of reagents of increasing reactivity in the dissolution process. The metal species extracted may then be associated with a specific chemical pool. These reagents are known to provide a means of estimating potentially *available* metal concentrations (Kheboian and Bauer, 1987). Most include up to five extractants with the following general properties:

1. Cation-exchanging extractants: these are solutions (0.05 to 1 mol dm^{-3}) of either sodium or ammonium acetate (adjusted to pH 7) or solutions of magnesium chloride, calcium chloride or sodium nitrate. The solutions act by displacing the ions from ion-exchangeable sites on the sample.
2. Carbonate-dissolving extractants: an example is a 1 mol dm^{-3} solution of acetic acid-sodium acetate at pH 5, which serves to dissolve a metal carbonate mineral phase.

3. Iron-manganese extractants: they dissolve oxyhydrates of iron and manganese and liberate other co-precipitated metal ions and oxides, using typically a solution of 1 mol dm^{-3} hydroxylamine and 25% v/v acetic acid.

4. Organic and sulphide bound metals: strong oxidising agents such as acidified hydrogen peroxide or sodium hypochlorite or sodium pyrophosphate are used to oxidise organic matter, releasing organically bond metal ions. Sulphides are very difficult to extract quantitatively.

5. Residual fraction: a strong acid extractant such as nitric acid (conc.) is used for dissolving silicates or minerals that have not been attacked by the milder reagents (Van Loon and Barefoot, 1992).

The exchangeable fraction and to a lesser extent the carbonate and organic fractions are generally considered to be the ones which constitutes the immediate nutrient reservoir in the soil solution. The biological significance and plant uptake of an element mainly depends upon its mobility. Thus the mobile fraction of an element in the soil may be defined as the sum of the soluble amount in the liquid phase and an amount retained by the solid phase, which can be transferred to the liquid phase (Kiekens and Cottenie, 1982). Extracting soil samples with nitric acid might be of interest to characterize unknown soils and provide useful information concerning their potential trace element status. In view of planning international programmes, unification of soil extraction would largely facilitate comparison and increase efficiency.

The validity of sequential extraction procedure has been questioned by a number of authors due to the lack of a standard set of reagents. The lack of a rigid test procedure makes interlaboratory comparison of metal fractionation in different soils difficult, and can give rise to inconsistencies and anomalies when comparing test data. Furthermore, nonselectivity of some extractants and trace element redistribution among phases during extraction are also problems associated with sequential extraction procedures. The analytical results are moreover influenced by the operating modalities such as soil/solution ratio, which may vary from 1/2 to 1/25. Nevertheless, such procedures do provide a valuable insight into the distribution of metals in contaminated soils, and can be used to explain the changes that occur in metal bioavailability in soils which have been the subject of *in situ* remedial action.

5.2. Metal bioavailability and speciation.

According to the theory of 'plant nutrient uptake', ions in soluble forms are more easily taken up by plants. Heavy metals in exchangeable forms have the highest solubility to give the highest potential bioavailability in contrast to the other chemical forms. However, heavy metals associated with carbonates are also easily released to solution when the soil pH is sufficiently low to dissolve the carbonates. Metabolic products of plant root, such as carbonic acid and other acid materials which are secreted during root physiological activities, would lower the pH of the rhizosphere to promote plant absorption of heavy metals from the carbonate form (Xian and Shokohifard, 1989). Massey (1972) reported that with lower soil pH, soluble heavy metal concentrations in the soil were increased, leading to uptake by plants (Xian, 1989). Heavy metals in the other chemical forms, such as iron-manganese oxides and organics with very low solubility and high stability for biological activity, would not have direct bearings on their uptake by plants (Asami *et al.*, 1995).

Exchangeable metals are held through electrostatic attraction on the exchange sites of the surface and interface of negatively charged inorganic and organic particles of the soil. Metals in this group are considered to be nonspecifically adsorbed and ion-exchangeable, i.e., they can be replaced by competing cations. The predominance of metals in contaminated soils in this fraction can lead to crop contents that can greatly exceed tolerable values for human and animal food stuffs.

The retention mechanism of heavy metals in soils changes according to the difference in speciation of the heavy metals and is influenced by soil solution pH. At higher pH values (above pH 8), metals will tend to precipitate and form hydroxy species, especially lead and copper. This enhances the retention of heavy metals in an hydroxide phase. It might be easier for heavy metals to form hydroxy species in the presence of soil particles because of the fact that the soil surfaces provide good nucleation sites where precipitates can grow at a faster rate.

The more soluble fractions, namely the exchangeable (ammonium acetate-extractable) and that associated with organic matter (hydrogen peroxide-extractable) are considered more "potentially available" to plants (Soon and Bates, 1982) because they are more likely to be released from the solid phases into the soil solution.

In contrast, the metals associated with the "residual" phase are not able to be released and may constitute the background level of metals in soil (Legret, 1993).

The residual fraction is generally considered to be within the lattice of silicate minerals and can become available only after digestion with strong acids at elevated temperatures. The residual materials consist of silicates and other resistant materials, and determination of the metal associated with this fraction, which is not considered to be significantly large, is important in completing mass-balance calculations (Yong *et al.*, 1993).

Associated changes in bioavailability may produce large increases or decreases in the risks of potentially toxic metals to crops, animals or man. It is therefore very important to establish what might happen to the chemical forms of metals present in such soils (Salomons and Forstner, 1980).

In the soil, the elements are stored in different ways and only those fractions which are soluble or may be solubilized can enter into a biological cycle. Therefore it is important to distinguish between the total quantities and the amounts which can be transferred into more mobile forms.

The distribution of an element between these forms is governed by the equilibrium constants of the corresponding reactions of precipitation and dissolution, complexation and decomplexation, adsorption and desorption. Equilibrium displacements may occur as a consequence of output of elements by plant uptake or by leaching, input of elements by manuring, changing water content (dilution or concentration) and pH changes. The main part of a trace element is present in the solid phase where it is incorporated in minerals and in precipitates. Due to competition with calcium, potassium and magnesium, only small quantities remain adsorbed and besides the free ions in solution, variable amounts may be bound in organo-metal complexes (Kiekens and Cottenie, 1982).

The mobility and the bioavailability of trace elements are related to the biological activity and the physicochemical properties of the soil. Heavy metals in ionic form, or complexed by organic materials have a very different nature and strength of bonding and therefore will react differently with the soil surfaces, so that the amount of these elements in bioavailable forms will greatly differ among different types of soils.

Field and laboratory studies have established relationships between metal speciation, availability and toxicity. The metals may transfer from contaminated soils into the growing plants and may retard the growth of plants and the growth of soil microorganisms. The bioavailability of metals in soil is determined by characteristics of both the soil and plants (Gupta and Aten, 1993).

5.3. A review of sequential extraction procedures.

Two different approaches can be discerned to characterise the pollution status of soils. The first is a single reagent leaching test, using an extractive reagent which is considered to be able to extract the bioavailable metal fractions. The use of single chemical extractants has been quite effective in evaluating the availability of soil trace elements which are essential for plant growth (West, 1981). Typical examples include the use of deionised water on different types of soil to examine soluble concentrations of copper, cadmium, nickel, zinc and lead (Petruzzelli *et al.*, 1981) and cadmium (Alloway *et al.*, 1979) and also iron, copper and manganese (Miller *et al.*, 1986). The second approach involves the use of sequential extraction techniques to distinguish between the different physico-chemical states of metals. This can give detailed information on the origin, mode of occurrence, biological and physicochemical availability, mobilisation and transport of trace metals (Tack and Verloo, 1995).

The metal species in soil can be defined as: (a) functional e.g. 'plant-available species'; (b) operational, according to the reagents or procedures used in their isolation or, most specifically, (c) as particular compounds or oxidation states of an element (Alloway, 1990). Operationally defined speciation often involves the use of single or sequential extractants to release metal species associated with particular soil phases. In sequential extraction, the sample is treated with a succession of reagents intended to specifically dissolve different soil phases, but the reagents used in sequential extraction procedures may not be sufficiently specific to dissolve exclusively the 'target' phases. Furthermore, results obtained from different laboratories can vary widely when different extraction schemes and experimental conditions are used as there appears to be only a general consensus on extraction schemes. Nevertheless, useful information has been gained from such studies (Davidson *et al.*, 1994).

Speciation reflects the flux of metal species in a certain medium, which contains both accelerating and inhibiting factors and processes. Such influences comprise the effects of pH-changes, redox reactions, inorganic and organic complexation and precipitation, and also microbially mediated species transformations such as biomethylation. Physical processes include adsorption, sedimentation and filtration. Biological barriers are often associated with membrane processes, which can limit translocation of metals. "Complexation" in its various forms can both inhibit and accelerate metal fluxes, particularly in biological systems consisting of different types of membranes (Forstner, 1993).

To understand the factors responsible for the elemental concentrations and to predict their possible evolution in a natural system, it is necessary to have exact information on the physico-chemical forms in which heavy metals exist in sediments and suspended matter. This will lead to a greater appreciation of their possible impact on plant well being and human health (Latouche *et al.*, 1993).

The value of selective sequential extraction analysis of heavy metal retention in soils can be seen in respect of their "partitioning". Despite some limitations, such as not being able to identify exactly the total proportions of heavy metals retained by the different soil constituents or as different phases in the soils studied, sequential extraction procedures are useful for assessing the relative importance of chemical forms that may be present in soils (Xian, 1989). Additionally, its usefulness in providing input to the evaluation of the capability of a particular type of soil and its potential for attenuating heavy metal contaminants in the leachate should not be discounted (Yong *et al.*, 1993).

Sequential extraction procedures can be used to identify the major metal species present in soils. In this way different mobilisation patterns are obtained which are characteristic for the behaviour of the elements under study. A summary of the extractants which have been used for each species are given in Table 5.1.

In the "exchangeable fraction" the metals are considered to be adsorbed and ion exchangeable. Release of the metals physically adsorbed on clay or soil particles surfaces can be accomplished by using a solution with excess cations. The "carbonate fraction" contains the metals which are precipitated or co-precipitated. These can be released by the use of a mild acid. In the "iron-manganese oxide fraction" the metals are specifically adsorbed or co-precipitated and are released by reduction. In the "organic fraction" the metals are complexed and adsorbed and are available through oxidation. Metals in the "residual fraction" are only available by digestion with strong acids (Clevenger, 1990). Each extractant represents a different level of availability and are described below. Water extracts the soluble fraction of metals; the potential asimilable fraction is extracted by acetates, whereas the exchangeable fraction can be extracted by neutral salts, such as K_2SO_4, KCl, NaCl, Na_2SO_4 etc. All these three fractions represent potentially bioavailable metal fractions for plant uptake.

The exchangeable fraction consists of metals retained by the matrix of the soil by ionic attraction or by means of Van der Waals forces (Petruzzelli *et al.*, 1994). Ammonium acetate is the preferred extractant, where the ammonium ion readily displaces the metal ion. In addition, it has been suggested that ammonium acetate removes chromium and zinc from ionic sites on organic matter and also from iron oxides; this extractant can also dissolve oxide

coatings in the hydrous oxide fraction of soils, and ammonium chloride or acetate can dissolve or complex transition metals from coatings on sediment grains. It has also been added to other stronger extractants to prevent undesired readsorption of the trace metals released by stronger extractants (Del Castihlo and Rix, 1993). Although ammonium acetate (1M) is not entirely selective, it nevertheless permits the extraction of metals adsorbed on the exchange sites of the humic acid and clay matrix of soils, thus giving a general picture of the availability of the metals (Hahne and Kroontje, 1973).

Significant correlations between ammonium acetate (1M) extractable cadmium concentrations in radish and lettuce (*Lactuca sativa L.*) grown on contaminated soils was reported by John *et al.* (1972) and the same extractant was also favoured by Andersson and Nilsson (1974) to determine plant available cadmium from contaminated soils. Andersson (1975) reported that ammonium acetate (1M; pH 4.8) was a more effective extractant for lead rather than some other commonly used reagents (see Table 5.1.). Haq *et al.* (1980) found the best correlation between zinc concentration in Swiss chard (*Beta vulgaris L.*) and zinc extracted by ammonium acetate (1M; pH 7.0) from metal-contaminated soils. Similarly, others have reported significant correlations between exchangeable forms of zinc and plant zinc concentrations (Le Claire *et al.*, 1984; Sims and Kline, 1991).

Cottenie and Verloo (1984) have suggested that ammonium acetate is only suitable for extracting metals from contaminated soils, although it is highly likely that in non-polluted soils metal concentrations are below the limits of detection of typical analytical instrumentation (such as ICP and AAS), and is not a consequence of the inability of ammonium acetate to extract the metals.

Heavy metals adsorbed or complexed by organic materials can be extracted by hydrogen peroxide solution, which is also able to dissolve heavy metal carbonates and, partially, some sulfides (Petruzelli *et al.*, 1994). The readsorption of released metal ions onto ion exchange sites can be prevented or reduced by lowering the pH of the solution, although this will be to the detriment of selectivity. Ammonium acetate has been added to hydrogen peroxide in order to stop the undesired readsorption of the trace metals released after this stage of sequential extraction procedures.

Organically bound metals incorporate those forms that are complexed, chelated, or adsorbed to organic matter in addition to components of living cells, their exudates, and a spectrum of degradation products (Wildung *et al.*, 1979). They are exchangeable only by other cations with higher affinities for the adsorption/complexation sites (Viets, 1962) and

References for Table 5.2.

1 = Tessier *et al.*, 1979.

2 = McGrath and Cegarra, 1992.

3 = Latouche *et al.*, 1993.

4 = Forstner, 1993.

5 = Miller *et al.*, 1986.

6 = Qiang *et al.*, 1994.

7 = Elliott *et al.*, 1990.

8 = Clevenger, 1990.

9 = Salomons and Forstner, 1980.

10 = Pierzynski and Schwab, 1993.

11 = Emmerich *et al.*, 1982.

12 = Chang *et al.*, 1984.

13 = Rapin and Forstner, 1983.

14 = Marcus and Sahibin, 1990.

15 = Singh and Narwal, 1984.

16 = Bell *et al.*, 1991.

17 = Jones, 1987.

Table 5.1. Review of Sequential Extraction Procedures

FRACTION	REAGENTS	REFERENCES																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	TS
Soluble	H_2O						•				•	•	•			•			
Exchangeable	$MgCl_2$ (1M, pH = 7.0)	•					•	•	•									•	
	$CaCl_2$ (0.1M)		•																
	$Ca(NO_3)_2$ (0.1M)					•											•		
	KNO_3 (0.5M)										•	•	•		•				
	NH_4OAc (1M, pH = 7.0)				•					•				•		• *			•
	DTPA (0.5M) + $CaCl_2$ (0.1M) + $(CH_3\text{-}CH_2\text{-}O)_3N$ (pH = 7.3)															•	•		
Acid soluble	NaOAc / HOAc (1M, pH = 5.0)	•			•		•	•	•				•						
(Bound to carbonates)	Na_2EDTA (0.05M)		•								•	•	•		•				
Reducible	$NH_2OH.HCl$ (0.04M) + 25% (v/v) HOAc (pH = 2.0)	•		•	•				•					•			•	•	
(Bound to Fe-Mn oxides)	$NH_2OH.HCl$ (0.01M) + HNO_3 (0.1M)					•				•							•		
	$(NH_4)_2C_2O_4$ + 0.1M $H_2C_2O_4$					•		•											
Oxidizable (Organically)	30% H_2O_2 (pH = 2.0) + HNO_3 (0.02M)	•			•				•					•				•	
+	30 % H_2O_2 (pH = 2.0)			•					•										
Sulphide bound	30 % H_2O_2 + NH_4OAc (1M, pH = 2.0)									•									•
	30 % H_2O_2 (pH = 2.0) + NH_4OAc (2M) in 20% (v/v) HNO_3	•			•									•				•	
	NaOH (0.5M)		•								•	•	•		•				
	$Na_4P_2O_7$ (0.1M)							•											
Residual	$HF/HClO_4/HCl$	•		•						•									
(Residual/Silicate)	HCl/HNO_3 (4:1 v/v)		•			•										•			
	HNO_3 (conc.)				•				•		•	•	•	•	•	•		•	•
	HCl (0.1M)														•	•			

Note: *pH = 4.8 not 7.0. TS= This study.

have generally been measured by extraction with chelating agents (Cox & Kamprath, 1972). At low pH, the organic compound-metal complexes are strongly adsorbed onto the adsorption substrates, particularly amorphous metal oxides, therefore enhancing metal adsorption (Jenne, 1977). Besides hydrogen peroxide, other reagents (e.g., potassium pyrophosphate, sodium hypochlorite) have been used for the oxidation of organic matter.

Organic matter has a significant impact on copper availability, and many studies have shown the greater ability of copper to form complexes with organic matter than zinc or manganese (Van Dijk, 1971; Mc Bride, 1978).

The residue remaining after the preceding extractions consists essentially of detrital minerals and resistant sulphides. Treatment with concentrated acid reagents results in complete dissolution. The available amounts of metals in a soil do not correspond with its total content and also, total chemical analysis is not necessarily indicative for the biological activity of polluting elements and therefore, more distinctive analysis is necessary to characterize their behaviour (Kiekens and Cottenie, 1982).

5.4. Speciation Experiments

The reagents used for the following have been chosen after reviewing several different sequential extraction methods (see Table 5.1.). Reagents utilised were chosen on the basis of their selectivity and specificity towards particular physicochemical forms.

5.4.1. Preparation of reagents

i) NH_4CH_3COO: (77.08 g, A.R.) was dissolved in deionised water and transferred to 1 litre volumetric flask and diluted to near volume. The pH of the solution was adjusted to 7.0 by adding a few drops of 1 M acetic acid and the solution was diluted to volume. The solution was then stored at 4°C.

ii) H_2O_2: (30%, A.R.) was purchased from Aldrich and was adjusted to pH 2 with HNO_3.

iii) HNO_3: (conc., A.R.) was purchased from Aldrich.

5.4.2. Preparation of soil samples

The soil samples collected from three main sources of metal pollution: sewage sludge (Gateacre), smelting activities (Prescot) and mining (Trelogan) (see Chapter 4 for details)

were dried at 80°C in an oven for 48 hrs., then ground in a mortar and sieved through a 0.5 mm plastic sieve. Zeolites P, 4A and Y obtained from Crosfield Chemicals, Warrington were used as received and were added to 100 g of dried soil in the ratios 0.5%, 1% and 5% w/w. The amended moist soils were then stored in plastic bottles at 4°C for one month before being used for experiments.

5.4..3. Extraction Procedures

5.4.3.1. Stage 1: Ammonium Acetate

Each of the soil samples (1.0000 g, Section 5.4.2.) were weighed into 50 cm^3 centrifuge tubes. To these were added NH_4OAc (20 cm^3, 1 M, pH 7) and the mixture shaken vigorously. After two hours at ambient temperature (ca. 20°C) the aqueous extract was separated from the soil residue by centrifugation (4000 rpm for 10 mins.). The supernatant was decanted into a polyethene bottle and stored at 4°C before being analysed for total metal content.

5.4..3.2. Stage 2: Hydrogen Peroxide

H_2O_2 solution (20 cm^3, 30%, pH 2) was added slowly to the residue from step 1, and carefully transferred to a soda glass quickfit round bottom flask fitted with a water condenser. The mixture was gently heated (ca. 80°C) for two hours with occasional agitation.

After this time, the mixture was allowed to cool, and NH_4OAc (20 cm^3, 1M, pH 7) was added to the mixture. The resulting mixture was heated for a further two hours, and after cooling the aqueous extract was separated and collected from the soil residue as above.

5.4.3.3. Stage 3: Nitric Acid

The residue from stage 2 was dried and weighed. HNO_3 (conc., 20 cm^3) was added to the soil residue and the mixture heated at 80°C for two hours. After cooling, the mixture was filtered and the residue discarded. The filtrate was diluted appropriately before analysis.

5.4.3.4. Analysis

The supernatant/filtrate from each sample was filtered through GF/C fibre glass filter paper prior to analysis by AAS. Standard Cu, Cd, Zn and Pb solutions were prepared in either NH_4OAc solution (1M) or HNO_3 (20%) to overcome any interference from the matrix. The extracted samples were diluted appropriately with deionised water before analysis by AAS. All solutions were kept at 4°C until analysis. Each solution was analysed in triplicate using standards in a similar matrix. Blanks were analysed in the same way.

5.5. Results and Discussion.

The effects of amending polluted soils with synthetic zeolites on the fractionation of cadmium, copper, lead and zinc were evaluated using a sequential extraction method on experimental soils (see Section 5.4.2.).

Sequential extraction procedures have been used to identify the chemical forms of heavy metals in the three soils studied (lead/zinc mining Trelogan site, copper refinery Prescot site and sewage sludge treated Gateacre site) and the changes associated with metal speciation on addition of each of the three zeolites: P, 4A and Y at different concentrations. Each zeolite was applied at rates of 0.5%, 1% and 5% w/w. The three extractants used for the fractionation of metals in these soils were: ammonium acetate for the soluble and exchangeable metals, hydrogen peroxide for the organically and sulfide bound metals and nitric acid for the residual metals. Untreated soil samples served as controls.

The total amount of copper, cadmium, zinc and lead in the soils and their distribution in the three fractions depended on total metal content, soil type, and soil properties (i.e. pH and percentage of sand, clay, organic matter and carbonates). There are clear differences in the proportion of each metal that was present in each of the three chemical fractions (Appendix 1).

The differences in extractability which have been observed depended on the zeolite used and the metal being extracted. The presence of all types of zeolite resulted in a significant reduction of exchangeable metal ions. After incubation of the polluted soils with synthetic zeolites, the amount of metals extracted by ammonium acetate was significantly decreased (31.4% - 72.4%) in comparison to the unamended soil (Fig. 5.1. and 5.2.). This is of great importance for the use of zeolite application in agrotechnical amendments, as heavy

metals extracted by this method constitute an exchangeable form, and are consequently potentially available for plant uptake.

5.5.1. Ammonium acetate extract.

Ammonium acetate (1M, pH 7) was used to liberate adsorbed and ion-exchangeable metals held through electrostatic attraction on the exchange sites of the negatively charged soil surface. The acetate ion complexes released metal ions, thus retaining them in solution and reducing the possibility of precipitation. Ammonium acetate is an extractant which does not alter the natural pH; it therefore reflects the concentration of trace metals in the soil solution and the readily exchangeable fraction, both strongly influenced by soil pH (Sauerbeck and Styperek, 1985).

The percent proportion of exchangeable metals varied independently of the soil characteristics, such as pH, soil type, organic matter content and heavy metal concentrations. As a general trend, increasing zeolite concentration from 0.5% up to 5% resulted in more exchangeable metals being trapped in the zeolite cages, thus reducing their bioavailability to plants (Fig. 5.2. a.- c.).

For zeolite Y, the reduction of the heavy metal content for Trelogan soil between 31.4% and 51.3% depended on increasing the amount of zeolite Y used (Fig. 5.1.a.). For Prescot soil, the percent reduction of ammonium acetate extractable metal varied between 43.8%- 67.46% (Fig. 5.1.b.), and for amended Gateacre soil, between 33% and 58% (Fig. 5.1.c.). For all three soils, zeolite Y showed smaller heavy metals sorption properties in comparison to the other zeolites (see Fig. 5.1. a. - c.). This is in agreement with the ion-exchange isotherms obtained for zeolite Y (see Chapter 3), which indicated a lower exchange capacity for this zeolite.

In the case of application of zeolite 4A to the three contaminated soils, the concentration of heavy metals in the exchangeable fraction was reduced between 40.3%-70.2%.

Even greater sorption properties in relation to heavy metals were apparent for zeolite P. For example, addition of zeolite P 5% reduced the amount of heavy metal extracted by ammonium acetate (1M) from Prescot soil by 72.37% compared to Prescot soil without zeolite P.

Figure 5.1. Concentration of all metals (m moles) in zeolite-amended soils.

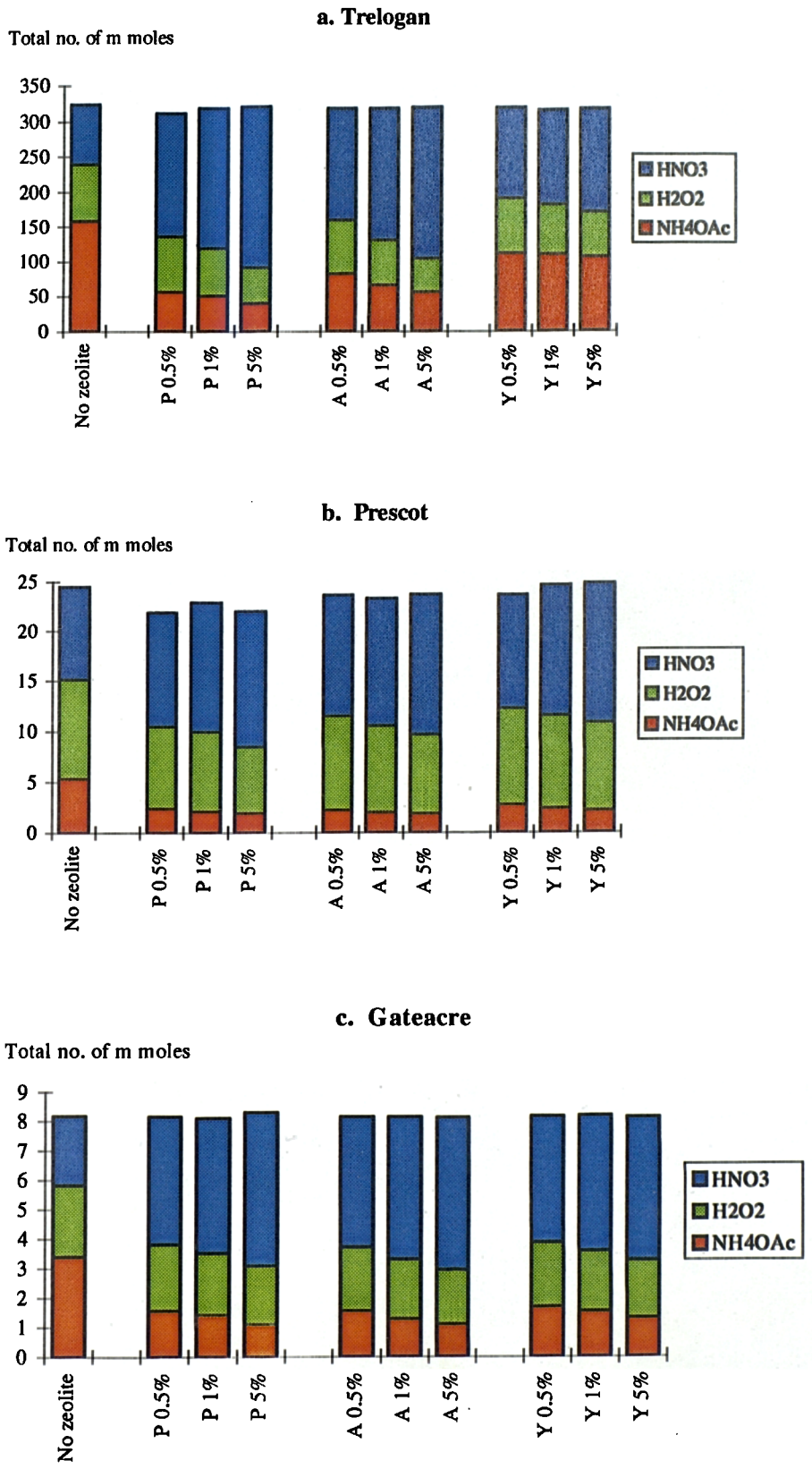
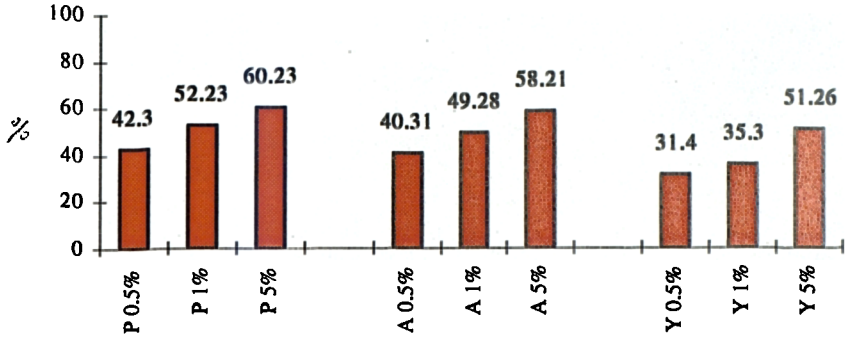
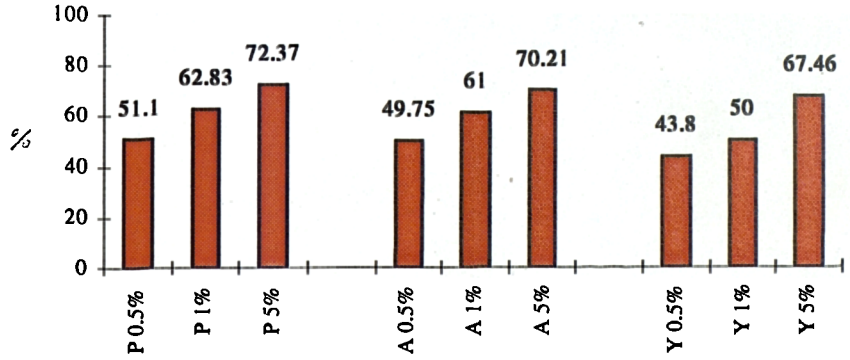


Figure 5.2. Percentage reduction of ammonium acetate extractable metal in zeolite-amended soils.

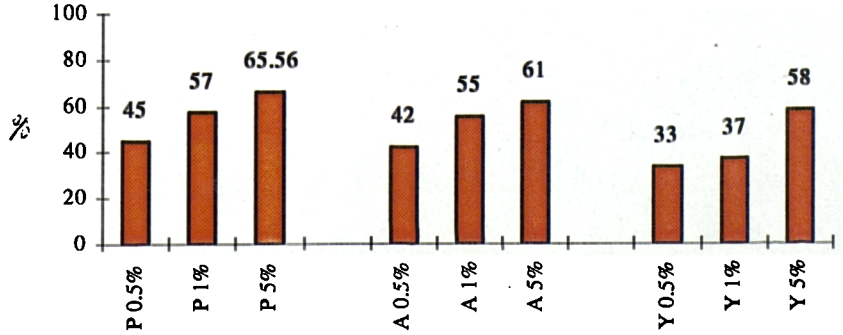
a. Trelogan



b. Prescot



c. Gateacre



While summing up the above results, it can be stated that the best reduction in the exchangeable heavy metal content in polluted soils was 60.23% for Trelogan soil, 72.37% in the case of Prescot soil and 65.56% for Gateacre soil with 5% zeolite P amendment.

For the ammonium acetate-exchangeable fraction, the zeolite efficiency was significantly higher for cadmium and zinc than for copper and lead. These observations are confirmed by data presented in Tables 1.1. - 1.4. in Appendix 1.

In general, cadmium in contaminated soils is in an "exchangeable" form (i.e. extracted by ammonium acetate in this case) (see Appendix 1 - Table 1.2.), and this relative ease of exchange must be considered in the light of both the mobility of this metal in soil horizons and the uptake of cadmium by plants (Legret, 1993).

Similar sequential extraction studies of the effects of synthetic zeolites showed that 78% of zinc, 70% of copper and 68% of lead are not available for plants when contaminated soils have been amended with zeolite 4A (Gworek and Borowiak, 1991). The investigations carried out by Gworek and Borowiak (1991) have shown that sewage sludge incubated with synthetic zeolites 13X and 4A resulted in a reduction in the ammonium acetate fraction by approximately 70% lead, 57% copper, 53.5% nickel, 67.5% zinc and 61% cadmium.

The evaluation clearly shows that in spite of the fact that the metal concentrations in ammonium acetate extracts are lower than in other extracts (Fig. 5.2. a.-c.), ammonium acetate fulfils most of the prerequisites and is one of the best available choices to predict biorelevant metal concentration in soils and indicates that it is particularly suitable for risk assessment of metals in contaminated soils (Gupta and Aten, 1993).

5.5.2. Hydrogen peroxide extract.

Organic-metal complexation, which involves simple complex formation and/or chelation, is a major mechanism responsible for the association of heavy metals with organic matter. Hydrogen peroxide (30%, pH 2) was used to extract heavy metals adsorbed or complexed by organic materials. The three soils under study contained different amounts of organic matter (Chapter 4). Trelogan has the smallest percentage of organic matter (3.5%), followed by Gateacre with 11.7% organic matter, whereas Prescot has the highest organic matter content of 27.3%, therefore having more metal complexed by available ligands. Fig. 5.2.b. shows that a high proportion of metal (ranging from 6×10^{-3} - 8.53×10^{-3} m moles) was detectable in the hydrogen peroxide oxidizable fraction of Prescot soil.

The four metals differ in their binding forms and their mobility. The different behaviour of the four elements agrees with their adsorption behaviour and coordination chemistry.

Many comparative studies have shown that soluble organic matter forms much stronger complexes with copper than with the other three metals. Soil organic matter (whether soluble or insoluble) is heterogeneous; copper being adsorbed on a number of sites of various binding strengths, the strongest being filled first (McGrath *et al.*, 1988). The main binding forms of copper, and also lead, are association with carbonates or iron oxides and especially in the case of copper, complexation by humic substances. Cadmium is particularly adsorbed in the diffuse layer of the cation exchanger or associated with carbonates, which results in a relatively high plant availability. In the soil solution, hydrated ions are the main species of cadmium and zinc, whereas copper is almost exclusively complexed by dissolved organic carbon (DOC). As hydrated ions are preferentially taken up by plants, the availability of cadmium and zinc is greater than that of copper and lead.

All the metals occurred in significant amounts in the organically bound form, with the majority of copper and lead being present in this form (see Appendix 1 - Tables 1.1. and 1.4.). Even in Trelogan soil, where the fraction of the organic matter was low (3.5%), a significant amount of the total soil copper was organically bound.

An enrichment amount of lead was found in the organic fraction as compared with cadmium and zinc where the organic fraction was lower with respect to other fractions. This is due to the fact that lead has strong affinity to humic acid (Takenaga and Aso, 1975) so usually combines with organic matter to form stable lead-organic complexes. The exchangeable fraction had smaller amount of lead suggesting that the solubility and mobility of this element was lower in the soils (see Appendix 1 - Table 1.4.).

5.5.3. Residual fraction.

To ensure partial decomposition of the silicates, nitric acid (conc.) was used for the residual metal fraction. Without exception, metal concentrations found in the residual fraction are higher than those observed in any of the preceding extractions (see Appendix 1 - Tables 1.1.-1.4.). The concentration of residual metals increased after the addition of zeolites to all the soils studied because the exchangeable metals and, to a smaller extent, the organic-bound metals, became trapped in the zeolites.

5.6. Conclusions

The exchangeable form is the most important form for heavy metal uptake by plants. The results of this study confirm that potentially bioavailable heavy metals are strongly controlled by their chemical forms and related to solubility. These results are summarised in Fig. 5.1., with zeolite Y having a lower efficiency which is in accordance with its lower exchange capacity previously observed in the ion-exchange experiments (see Chapter 3).

A decreasing trend in the extractability of cadmium, copper, zinc and lead by ammonium acetate with increasing zeolite concentration was observed and, although in different degrees, it was consistent for all three synthetic zeolites studied. The percentage reduction of ammonium acetate extractable metals varied from 42.3% to 72.4% for zeolite P, for zeolite 4A - between 40.3% and 70.2%, and for zeolite Y ranged from 31.4% to 67.46% (Fig. 5.2. a.- c.).

The heavy metals in zeolite-treated soils changed their chemical forms following zeolite incorporation. The percentage of heavy metals in the ammonium acetate-extracted fraction for all metal elements examined (and, in some cases, also for the hydrogen peroxide-extracted fraction for copper, cadmium, zinc and lead) decreased significantly with zeolite treatment. Therefore the plant available soil fractions of zinc, cadmium, copper and lead were significantly reduced by the addition of synthetic zeolites P, 4A and Y, with an equivalent increase in the amount of metal being recovered from the insoluble fractions only removable with strong acid extractions (see Fig. 5.1. a. - c.).

With respect to the heavy metal-soil interaction determined through sequential extraction, the amount of lead and copper held in the exchangeable position was found to be lower than the amount of cadmium and zinc. This reflects a stronger fixation of copper and lead with the organic matter (Schnitzer and Skinner, 1966). The mobilization patterns of zinc and cadmium indicate that ion-exchange is an important factor in the retention of these elements by the solid phase of the soil.

The strong association of copper with the oxidisable fraction of soil has been demonstrated in numerous studies (Sposito, 1983; McGrath *et al.*, 1988). In areas of low organic content, inorganic copper species, particularly copper carbonate, may become important and biologically significant, as carbonate complexation reduces the toxicity of copper (Langston and Bryon, 1984). The preference of copper for organic matter is supported by the high stability constants of copper complexes with organic matter (Irving and Williams, 1953).

The residual form also contained significant amounts of metals, with zinc having the lowest percentage present in this form. The residual form is considered the most stable form.

The distribution of trace elements between different forms in the soil is the result of several equilibrium reactions. Once incorporated into the soil, the forms of the metals will shift to more stable forms, so that the chemical equilibria of the soil will always be maintained. When the soluble fraction of the metals is leached from the soil due to acid rain or soil washing, some of the metals complexed by organic matter will shift towards the exchangeable sites and the metals in the residual form will also slowly shift toward the organic/exchangeable form. The possibility that the metals would continue to slowly shift toward the residual form with time should not be an unreasonable expectation, as the more stable solid phase form of a heavy metal will be, less likely to dissociate into the solution phase and become available for movement with the soil solution.

The simple extraction scheme used here successfully identified differences in the fractions according to the chemical nature of each metal, and clear differences between the fractions present in zeolite-treated soils compared to the unamended soils. When zeolite amended soils are fractionated, a smaller percentage of the total metals present is recovered in forms readily available for plant absorption (e.g., soluble, exchangeable, or sorbed forms).

The metal concentrations were statistically analysed following the same procedure described in Section 7.5. For statistical analysis, all data were previously expressed as a zeolite treatment : untreated control ratio. ANOVA followed by Tukey multiple comparison tests revealed significant differences ($P < 0.05$) between all zeolites at all concentrations, for all soils and metals for the ammonium acetate fraction. For the hydrogen peroxide fraction, significant differences ($P = 0.001$) were obtained for zeolites P and 4A at 1% and 5% versus untreated control. No significant differences between treatments were recorded for the residual fraction.

The results from the selective sequential extraction analysis reveal the importance of the effectiveness of synthetic zeolites with regard to heavy metal immobilisation. In order to estimate how realistically this procedure relates to actual events in the soil solution, the next step of this study involved the incubation of moist soils with zeolites for a longer period of time, and the soil solution analysed following standard procedures (Sanders *et al.*, 1987; Litaor, 1988; Grossmann & Udluft, 1991).

Because some metals (such as cadmium and zinc) are strongly fixed in the soil over a wide pH range, it is difficult to determine their long term displacement only by field observations. The water displacement method described by Sanders *et al.* (1987)

was used to estimate long term changes of the heavy metal content in the soil solution of zeolite-amended soils. To evaluate the durability of the metal immobilising effect of the soil treatment, the metal concentrations in the water leachates were measured every month over a period of three months after the soil treatment.

The soil extraction data has indicated that addition of synthetic zeolites to contaminated soils results in changes in the binding of heavy metals in soils. Therefore, synthetic zeolites would be promising for *in situ* immobilisation of heavy metals in contaminated soils. To understand the fate and distribution of metals in contaminated soils, a combination of field and pot experiments were initiated in which various plant species were grown in zeolite-amended soils in order to estimate their long-term efficacy.

CHAPTER 6.
SOIL SOLUTION STUDIES WITH SYNTHETIC ZEOLITES.

6.1. Introduction

From a strictly chemical point of view, a soil is a multicomponent, reactive system comprising solid, liquid, and gaseous matter, subjected to electromagnetic and gravitational fields. Soil chemistry focuses primarily on the aqueous phase - the soil solution - and investigates the processes which occur in it as a result of the actions of biological, hydrological, and geological agents. Thus the soil solution is a dynamic and open natural water system whose composition reflects the complicated reactions that can proceed simultaneously between an aqueous solution and a mixture of mineral and organic solids which themselves vary both temporally and spatially.

The principal chemical reactions of interest in soil solutions are soluble complex formation, oxidation-reduction, adsorption, ion exchange, and precipitation-dissolution. All of these reactions play an important role in the uptake of trace metals by plants (Sposito and Bingham, 1981).

The soil solution is the aqueous portion of soil that contains dissolved matter from soil chemical and biochemical processes and from exchange with the hydrosphere and biosphere. This medium transports chemical species to and from soil particles and provides intimate contact between the solutes and soil particles. In addition to providing water for plant growth, it is an essential pathway for the exchange of plant nutrients between roots and solid soil. Obtaining a sample of soil solution is often very difficult because the most significant part of it is bound in capillaries and as surface films (Manahan, 1994).

The importance of soil solutions in environmental studies was recognised long ago by Joffe (1933), who described the soil solution as the "blood circulation of the soil body". The chemistry of soil solutions provide important information regarding distribution of nutrients, their mobility and availability to plants (Sollins *et al.*, 1980) and it can also be used as a sensitive medium for calibrating and validating theoretical models of solute transport (Van de Pol *et al.*, 1977). Soil solutions also provide crucial data concerning the acid-neutralising capacity (ANC) of a soil system (David and Driscoll, 1984) and the magnitude and rate of movement of pollutants to groundwater (Fortescue, 1980).

Soil solutions provide information about the kinetics of solid-solution interaction *in situ* (Murali and Aylmore, 1980). McDowell and Wood (1984) used soil solution chemistry to asses the mechanism of podzolization and pedological control on dissolved organic carbon concentrations in stream water (Litaor, 1988).

The study of trace metal chemistry in soils which support crop plants is made complicated by the large number of reactions possible between trace metals and soil constituents and by the web of interrelations among trace metals and the macrocomponents of soil solutions which is created by these reactions. In a typical soil solution, there may be 10-20 different metal cations present (including trace metals) which can react with as many different inorganic and organic ligands to form 300-400 soluble complexes and up to 80 solid phases. In addition, redox, ion-exchange, and specific adsorption reactions occur (Sposito and Bingham, 1981).

Metals present in the soil solution can be free metal ions, soluble complexes with organic or inorganic ligands, or associated with mobile colloidal materials. Soil solution studies generally show that plant response to metals is correlated with the free metal ion activity. Therefore, one aspect of metal bioavailability is related to which factor or factors contribute to the activity of the free metal ion in the soil solution (Pierzynski *et al.*, 1994).

The chemical composition of the soil solution reflects the demand of soil biological processes and the solubility and ion-exchange equilibria between physical and biological components of the soil. The aqueous solution chemistry of metal ions play a central and dominant role in determining their interaction and effect on plants.

Two principles are used as guides to understand the overall features of metal uptake by plants from soil solutions. However, they cannot provide detailed information as to uptake mechanisms (Sposito and Bingham, 1981).

The Bonding Rule states that nutrient trace metals tend to form complexes through large decreases in entropy mediated by electrostatic interactions, whereas toxic trace metals tend to form complexes through large decreases in enthalpy mediated by covalent interactions.

The Selectivity Rule says that the relative selectivity for a given trace metal by a complexing ligand site which is involved in an uptake process is proportional to the product of the total concentration of the metal in solution with the stability constant for the metal-ligand complex.

Consideration of rates of release of heavy metals from soil exchange surfaces, in addition to their concentrations and those of interacting cations in soil solution, may be

necessary in order to asssess fully the plant availability of heavy metals from a wide range of soils.

The concept that the soil solution plays a critical role in controlling the availability of ions to plants is long established (e.g. Whitney & Cameron, 1903). As uptake of ions by plants occurs from the solution phase, it is heavy metals in this phase that have the greatest ecological significance as well as species which are easily solubilised. In solution, the toxicity of metals is proportional to the concentration of free, i.e. uncomplexed, metal ions. Complex formation with chelating ligands reduces the concentration of free ions and thus inhibits the metal toxic effects (Spencer and Nichols, 1983).

Soil-column studies by Tyler and McBride (1982) on the mobility of heavy metals (cadmium, copper, nickel and zinc) in several soils indicate that least mobility (i.e., transport) of metals was obtained in a mineral soil with a relatively high pH, CEC, and exchangeable base content. Therefore, soils with these characteristics will exhibit good retention capability.

Under field conditions, soils are subjected to pH changes either by farm management practices (e.g. liming, leguminous pastures) or other processes (e.g. acid rain). Similarly the chemical composition and ionic strength of the soil solution will vary depending on the nature of the parent material, the weathering environment, mineral constituents, soil amendments and management practices. The ionic strength of soil solutions ranges from < 0.005 M in soils from tropics (Naidu *et al.*, 1991) to > 0.10 M in the less weathered soils from temperate climates (Edmeades *et al.*, 1985) or near fertiliser granules in the microenvironment. The effect of ionic strength on anion and cation adsorption varies with pH. The effect of ionic strength on metal sorption is often attributed to changes in soil-suspension pH (Barrow & Ellis, 1986) through its effect on the diffuse double layer and consequent changes in the ions present (Naidu *et al.*, 1994).

Many different methods have been developed to obtain soil solution samples, either *in situ* or in the laboratory (Litaor, 1988; Grossmann & Udluft, 1991). However, no single method can claim to be generally valid and to obtain the "true" unaltered soil solution. This is partly due to technical limitations (Litaor, 1988), but mainly because of the temporal and spatial variability of soil-solution properties on a macro- and microscopic scale (Sposito, 1983). Despite these limitations, soil-solution data can provide valuable information on the dynamics of ions within the soil profile, and over time on the availability of nutrients or toxic ions to plants. The solutions closely represent the conditions in the soil to which the plant roots are exposed.

Three techniques for destructively extracting soil solutions are commonly used; each have their associated advantages and disadvantages and applicability to different soil types: miscible displacement (Adams, 1974), which utilises a displacing solution to push the soil solution out of a packed column; immiscible displacement by a water-insoluble organic liquid, with gravitational displacement by centrifugation (Kinniburgh and Miles, 1983); and extraction of solution already in the soil by centrifugation (Ross and Bartlett, 1990).

Experiments on the bioavailability of either macro-or microelements to plants, which are based on soil-solution parameters, should sample soil solution from the rhizosphere using non-destructive, low-tension methods like water displacement (Sanders *et al.*, 1987) in order to obtain a true indication of the conditions the plant roots are exposed to. However, such low-tension methods are probably limited to be used on light to medium textured soils, but they may fail on soil with a high clay content. Solution composition may change substantially over time, so soil solution would need to be sampled repeatedly during one experiment to give a realistic reflection of the overall conditions.

Soil is a very complex substrate, with many factors operating in it, such as sorption, chelation by organic matter or precipitation reactions. These factors are the cause of varying bioavailability in different soils, and total concentrations do not adequately characterise the risk of a given concentration in all soils, therefore measurement of the amount of metals in the soil solution which bathes plant roots and soil organisms is a better indicator of metal bioavailability. Measuring the speciation of metals in soil solution is more biologically relevant.

6.2. Experimental

The soil solution composition was determined using water displacement (Sanders *et al.*, 1987). Typically, 10.0 g of soil (which had been previously rehydrated, see below) was placed into a 30 by 2 cm glass column. Each column had a sintered glass base. The column was gently jarred during filling to partially settle the soil. The degree of soil compaction required for effective solution displacement had been previously determined by trial and error on separate samples. The column was placed in a suitable rack. Distilled water (100 cm^3) was added to the column and allowed to saturate the soil for two days in order to achieve equilibration of the added water with that already present in the soil. Following this saturation period, the stopcocks at the base of the soil columns were opened and the resulting leachate

solutions were eluted from each soil column. The first 50.0 cm^3 of leachate was collected in a beaker, and the remained discarded (Fig. 6.1.).

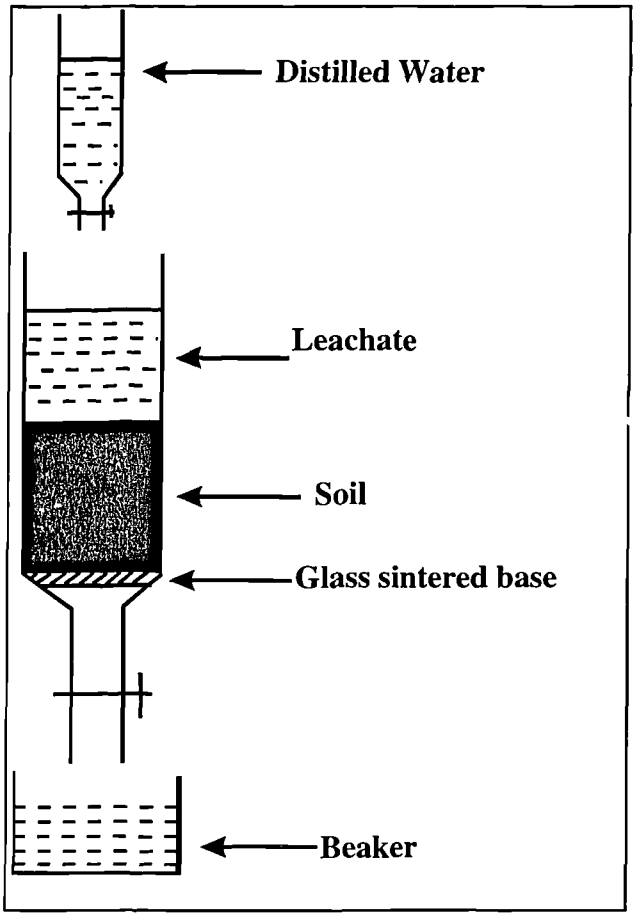
Soil samples collected from Trelogan, Prescot and Gateacre (see Chapter 4 for details) were ground in a mortar and then sieved through a 0.5 mm plastic sieve. The requisite amount of synthetic zeolite 4A, P and Y (0.50%, 1.00% and 5.00% w/w dry weight) was added to each soil and mixed. Untreated soil samples served as controls. Each soil was air dried; therefore, needed to be rehydrated, so that it was moist. The amount of distilled water added to each soil varied depending on soil texture and initial moisture content. To each 10.0 g of air dried soil: 2.6 cm^3 (Trelogan), 4 cm^3 (Gateacre) and 6 cm^3 (Prescot) of distilled water was added in order to re-hydrate the soils.

Two replicates of 250 g of each soil were incubated in a dark, cool room (4°C) for a period of three months and sampled at regular intervals. The room temperature was permanently recorded using a maximum-minimum thermometer. Additional control samples of Prescot and Gateacre soils amended with lime (calcium carbonate) were also incubated. To these soils, an appropriate quantity of lime was added to bring the soil pH to a value of 6.5.

The synthetic zeolites increased the soil pH (Tables 6.1. and 6.2.), therefore additional columns were packed with the three unamended soils and were leached with distilled water which had been adjusted to the same pH as the zeolite-amended soils. NaOH (0.1M) was used to increase the pH of the water. These allowed a more direct comparison of the effectiveness of synthetic zeolites in reducing the heavy metal availability to the plants.

Soil samples were taken from each of the control and amended soils at intervals of 0, 30, 60 and 90 days. The soil solution composition was determined by water displacement, and then filtered through fibre glass filter paper (Whatman GF/C) into polyethylene bottles. The time required for collecting enough soil solution for analysis varied between 8 and 12 hours. The pH of the leachate was tested immediately using a Radiometer PHM 85 precision pH meter fitted with a Radiometer GK 2401 C combination glass electrode. The collected leachate was subsequently analysed for copper, cadmium, zinc and lead using a Perkin Elmer SP9 Atomic Absorbtion Spectrophotometer. Precision was checked by triplication and blanks.

Fig. 6.1. Column Design Experiment



6.3. Results and Discussion

Determination of the chemical composition of the soil solution is an effective method to analyse the effect of zeolites, since the soil solution is a direct medium for the plant root, and the changes in its composition directly affect plant growth.

All the synthetic zeolites initially chosen as possible candidates for land remediation have been subjected to soil column tests. Soil columns were constructed to determine the immobilisation efficiency of amended soils in a dynamically-changing soil environment. The purpose of this work was to test the ability of the various amendments to remove leached metals from solution. Two aspects of soil conditions were examined in detail: pH control and the effect of the amendment on metal release into solution. As a measure of the performance of each amendment the pH and metal content of the leachate was recorded.

6.3.1. pH

The pH increase in the soil-zeolite mixture reflected the high pH values of synthetic zeolites: both the initial soil pH and the pH of the leachates increased with increasing zeolite concentration (Appendix 2). The zeolites increased the soil pH immediately after being mixed with the soils, but no further changes in the pH values were recorded after 30, 60 or 90 days.

Zeolites P and 4A increase the pH more than zeolite Y (Tables 6.1. and 6.2.). This is due to the lower exchange capacity of zeolite Y (Chapter 3).

Table 6.1. Initial pH of soil samples (5 g moist soil + 12.5 ml distilled deionised water) (n= 3).

Soil	Trelogan		Prescot		Gateacre	
Sample	Initially	Average of three months	Initially	Average of three months	Initially	Average of three months
Unamended	**7.2 (±0.4)**	**7.6 (±0.4)**	**3.3 (±0.6)**	**3.8 (±0.4)**	**5.0 (±0.2)**	**5.4 (±0.5)**
P 0.5%	10.2 (±0.3)	10.5 (±0.4)	5.4 (±0.5)	5.6 (±0.5)	6.2 (±0.5)	7.0 (±0.3)
P 1%	10.4 (±0.5)	10.6 (±0.5)	6.1 (±0.2)	6.4 (±0.4)	6.9 (±0.4)	7.6 (±0.3)
P 5%	11.3 (±0.4)	11.3 (±0.4)	7.0 (±0.4)	7.7 (±0.6)	8.4 (±0.4)	9.0 (±0.3)
A 0.5%	10.2 (±0.3)	10.6 (±0.3)	5.6 (±0.5)	5.4 (±0.4)	6.4 (±0.4)	7.0 (±0.3)
A 1%	10.6 (±0.5)	10.8 (±0.4)	6.2 (±0.5)	6.2 (±0.3)	7.2 (±0.4)	7.5 (±0.3)
A 5%	11.2 (±0.4)	11.3 (±0.4)	7.2 (±0.5)	8.0 (±0.3)	8.2 (±0.4)	8.0 (±0.4)
Y 0.5%	8.6 (±0.5)	8.8 (±0.4)	3.9 (±0.4)	4.2 (±0.5)	4.8 (±0.3)	5.4 (±0.4)
Y 1%	9.4 (±0.3)	9.6 (±0.4)	4.3 (±0.4)	4.7 (±0.3)	5.0 (±0.5)	5.4 (±0.3)
Y 5%	10.4 (±0.5)	10.6 (±0.4)	5.2 (±0.3)	5.3 (±0.3)	6.0 (±0.3)	6.3 (±0.2)
+ 1% CaCO3	-	-	5.0 (±0.4)	5.4 (±0.3)	7.0 (±0.2)	7.4 (±0.3)

Note: Figures in brackets represent standard deviation.

Table 6.2. pH of soil samples measured in the first 50 ml distilled deionised water which was leached through 10 g of moist soil column (n=3).

Soil	Trelogan		Prescot		Gateacre	
Sample	Initially	Average of three months	Initially	Average of three months	Initially	Average of three months
Unamended	**7.4 (±0.2)**	**7.6 (±0.1)**	**3.6 (±0.4)**	**4.4 (±0.3)**	**4.6 (±0.2)**	**7.3 (±0.3)**
P 0.5%	9.0 (±0.5)	8.8 (±0.3)	5.6 (±0.5)	6.4 (±0.3)	6.8 (±0.7)	7.3 (±0.4)
P 1%	9.4 (±0.3)	9.5 (±0.3)	6.9 (±0.2)	7.2 (±0.3)	6.8 (±0.5)	8.0 (±0.3)
P 5%	10.0 (±0.2)	10.0 (±0.4)	7.0 (±0.6)	8.3 (±0.3)	8.2 (±0.4)	8.8 (±0.3)
A 0.5%	7.6 (±0.7)	8.7 (±0.3)	4.8 (±0.5)	6.0 (±0.5)	5.6 (±0.5)	7.3 (±0.3)
A 1%	10.0 (±0.4)	9.7 (±0.2)	6.2 (±0.4)	7.2 (±0.3)	6.0 (±0.4)	8.0 (±0.3)
A 5%	10.2 (±0.6)	10.6 (±0.3)	7.2 (±0.3)	8.0 (±0.3)	7.8 (±0.3)	8.6 (±0.3)
Y 0.5%	8.0 (±0.9)	7.5 (±0.4)	4.1 (±0.5)	5.0 (±0.3)	5.2 (±0.4)	7.0 (±0.3)
Y 1%	8.0 (±0.5)	8.6 (±0.4)	4.4 (±0.5)	6.0 (±0.3)	5.4 (±0.4)	8.0 (±0.4)
Y 5%	9.0 (±0.9)	9.6 (±0.6)	5.0 (±0.3)	6.2 (±0.3)	6.6 (±0.5)	8.0 (±0.4)
+ 1% CaCO3	-	-	5.4 (±0.4)	6.0 (±0.4)	6.6 (±0.5)	8.2 (±0.4)

Note: Figures in brackets represent standard deviation.

Zeolites increase soil pH due to exchange of the Na^+ ions from their cages for the H^+ ions in the soil solution and therefore pH increases because less H^+ ions are present in the soil. Increasing soil pH causes the metals to precipitate. Therefore, to test the zeolites ability to decrease metal concentration by increasing the pH or by an ion-exchange process, calcium carbonate was added to increase the pH of the unamended soils. In addition, the unamended soils were leached with distilled deionised water having the same pH as the zeolite-treated soils.

6.3.2. Metal Content

The soil solution was sampled at regular intervals (0, 30, 60 and 90 days) to investigate whether zeolite addition was effective throughout the period of soil incubation, or whether changes in heavy metal content in the soil solution occurred.

All the values of the metal concentrations in the soil solution were compared to the metal content of the unamended soils which were leached with distilled water which had been adjusted to the same pH as the zeolite-amended soils.

Metal concentrations in the soil solutions are given in Tables 6.3.- 6.6. After 30 days, the incorporated synthetic zeolites decreased the soil solution concentrations of all the metals in the amended soils. No further change in soil solution composition was observed up to the ninety days test period. The metal content in the amended soils was reduced by 47% with zeolites P or 4A and only by 37.5% with zeolite Y and remained unchanged with time. The results in Tables 6.3.-6.6. also indicate that 1% calcium carbonate addition was less effective than the synthetic zeolites at immobilising heavy metals in Prescot and Gateacre soils: the zinc concentration in Prescot soil was 10 $\mu g.g^{-1}$ after adding 5% zeolite P and 25 $\mu g.g^{-1}$ when lime was added. 1.3 $\mu g.g^{-1}$ of cadmium was found in Gateacre-limed soil versus 0.6 $\mu g.g^{-1}$ in zeolite P-amended soil.

No further significant changes occurred in the concentration of copper, cadmium, zinc and lead in the soil solutions whether they were obtained 60 or 90 days after zeolite incubation. The metal concentrations in the soil solutions measured after three months of zeolite incubation were similar to those measured after one month (Appendix 3). Long-term stability tests with contaminated soils amended with synthetic zeolites showed that very little metal leaching occurred over a period of 90 days, indicating that the zeolite structures were

Table 6.3. Water extractable copper measurements for soil solutions (distilled deionised water leached through a 10 g soil column → 50 ml leachate) (n=3).

Soil	Trelogan		Prescot		Gateacre	
Sample	Initially	Average of three months	Initially	Average of three months	Initially	Average of three months
Unamended (pH = 6)	**0.60 (±0.20)**	**0.70 (±0.04)**	**53 (±3.6)**	**51 (±3.5)**	**1.7 (±0.3)**	**1.8 (±0.2)**
Unamended (pH = *)	**0.46 (±0.03)**	**0.50 (±0.04)**	**45 (±3.6)**	**46 (±3.3)**	**1.5 (±0.5)**	**1.4 (±0.2)**
P 0.5%	0.45 (±0.03)	0.40 (±0.03)	44 (±3.6)	31 (±2.3)	1.5 (±0.4)	1.0 (±0.3)
P 1%	0.48 (±0.03)	0.34 (±0.04)	43 (±2.6)	25 (±2.3)	1.4 (±0.1)	0.8 (±0.2)
P 5%	0.43 (±0.03)	0.30 (±0.02)	43 (±1.0)	23 (±3.0)	1.4 (±0.1)	0.7 (±0.0)
A 0.5%	0.44 (±0.02)	0.30 (±0.03)	45 (±4.3)	36 (±3.6)	1.4 (±0.3)	1.2 (±0.3)
A 1%	0.42 (±0.02)	0.32 (±0.04)	44 (±1.7)	26 (±3.2)	1.5 (±0.2)	0.8 (±0.2)
A 5%	0.43 (±0.03)	0.30 (±0.03)	42 (±4.4)	25 (±2.0)	1.4 (±0.4)	0.7 (±0.1)
Y 0.5%	0.46 (±0.01)	0.44 (±0.03)	46 (±1.7)	43 (±3.0)	1.6 (±0.2)	1.3 (±0.1)
Y 1%	0.45 (±0.03)	0.40 (±0.03)	43 (±2.6)	41 (±2.4)	1.6 (±0.3)	1.2 (±0.3)
Y 5%	0.42 (±0.03)	0.32 (±0.03)	41 (±3.6)	26 (±2.3)	1.5 (±0.3)	0.8 (±0.1)
+ 1% CaCO3	-	-	43 (±8.2)	40 (±3.0)	1.4 (±0.1)	1.2 (±0.2)

Note: Figures in brackets represent standard deviation; 6 = Initial pH (7.4 for Trelogan; 3.6 for Prescot; 4.6 for Gateacre);

* = Adjusted pH (10.0 for Trelogan; 6.5 for Prescot; 6.5 for Gateacre).

Table 6.4. Water extractable cadmium measurements for soil solutions (distilled deionised water leached through a 10 g soil column—> 50 ml leachate) (n=3).

Soil	Trelogan		Prescot		Gateacre	
Sample	Initially	Average of three months	Initially	Average of three months	Initially	Average of three months
Unamended (pH = 6)	**8.5 (±0.9)**	**8.2 (±0.8)**	**0.8 (±0.2)**	**0.8 (±0.1)**	**1.7 (±0.3)**	**1.7 (±0.3)**
Unamended (pH = *)	**7.0 (±0.9)**	**6.8 (±0.8)**	**0.7 (±0.1)**	**0.7 (±0.1)**	**1.3 (±0.3)**	**1.2 (±0.2)**
P 0.5%	6.8 (±0.3)	5.5 (±0.5)	0.7 (±0.0)	0.3 (±0.0)	1.3 (±0.0)	0.8 (±0.2)
P 1%	6.6 (±0.4)	5.0 (±0.7)	0.6 (±0.0)	0.3 (±0.1)	1.2 (±0.3)	0.7 (±0.1)
P 5%	7.2 (±0.3)	4.3 (±0.6)	0.6 (±0.1)	0.3 (±0.1)	1.2 (±0.0)	0.6 (±0.1)
A 0.5%	7.1 (±0.4)	5.6 (±0.7)	0.7 (±0.1)	0.5 (±0.0)	1.3 (±0.3)	0.8 (±0.0)
A 1%	7.0 (±0.6)	5.4 (±0.5)	0.7 (±0.1)	0.4 (±0.1)	1.3 (±0.3)	0.6 (±0.1)
A 5%	6.7 (±0.6)	4.2 (±0.6)	0.7 (±0.2)	0.3 (±0.1)	1.3 (±0.0)	0.5 (±0.1)
Y 0.5%	7.5 (±0.5)	6.6 (±0.4)	0.7 (±0.0)	0.6 (±0.1)	1.3 (±0.1)	0.9 (±0.1)
Y 1%	7.3 (±1.0)	6.0 (±0.6)	0.7 (±0.1)	0.6 (±0.1)	1.3 (±0.5)	0.8 (±0.1)
Y 5%	7.0 (±0.3)	5.0 (±0.5)	0.7 (±0.1)	0.4 (±0.0)	1.3 (±0.3)	0.7 (±0.2)
+ 1% CaCO3	-	-	0.6 (±0.2)	0.5 (±0.1)	1.3 (±0.2)	1.0 (±0.3)

Note: Figures in brackets represent standard deviation; 6 = Initial pH (7.4 for Trelogan; 3.6 for Prescot; 4.6 for Gateacre);

* = Adjusted pH (10.0 for Trelogan; 6.5 for Prescot; 6.5 for Gateacre.

6.5. Water extractable zinc measurements for soil solutions (distilled deionised water leached through a 10 g soil column → 50 ml leachate) (n=3).

Soil	Trelogan		Prescot		Gateacre	
Sample	Initially	Average of three months	Initially	Average of three months	Initially	Average of three months
Unamended (pH = 6)	**70 (±4.6)**	**70 (±5.0)**	**30 (±6.6)**	**28 (±4.7)**	**39 (±4.8)**	**39 (±4.8)**
Unamended (pH = *)	**65 (±2.6)**	**60 (±5.0)**	**28 (±2.6)**	**26 (±3.7)**	**38 (±10)**	**36 (±4.7)**
P 0.5%	63 (±3.0)	45 (±4.3)	28 (±4.4)	13 (±4.2)	37 (±4.4)	24 (±2.6)
P 1%	61 (±1.7)	40 (±4.8)	27 (±2.6)	12 (±2.3)	36 (±4.0)	21 (±3.0)
P 5%	60 (±4.4)	32 (±2.7)	26 (±1.0)	10 (±3.2)	36 (±5.3)	17 (±2.0)
A 0.5%	62 (±5.3)	48 (±4.0)	28 (±2.0)	14 (±2.6)	36 (±5.3)	23 (±3.5)
A 1%	62 (±2.0)	40 (±3.6)	27 (±8.2)	13 (±2.2)	38 (±2.0)	19 (±1.7)
A 5%	61 (±5.3)	33 (±4.4)	27 (±4.4)	11 (±1.9)	35 (±2.6)	16 (±2.0)
Y 0.5%	64 (±5.3)	58 (±3.2)	28 (±2.6)	20 (±4.6)	40 (±1.7)	30 (±3.0)
Y 1%	60 (±5.3)	50 (±3.7)	28 (±8.5)	18 (±2.0)	38 (±7.0)	24 (±2.3)
Y 5%	60 (±8.6)	40 (±4.4)	26 (±5.6)	12 (±2.2)	38 (±2.0)	21 (±2.4)
+ 1% CaCO3	-	-	26 (±1.7)	23 (±5.2)	36 (±3.6)	31 (±3.4)

Note: Figures in brackets represent standard deviation; 6 = Initial pH (7.4 for Trelogan; 3.6 for Prescot; 4.6 for Gateacre);

* = Adjusted pH (10.0 for Trelogan; 6.5 for Prescot; 6.5 for Gateacre).

Table 6.6. Water extractable lead measurements for soil solutions (distilled deionised water leached through 10 g soil column → 50 ml leachate) (n=3).

Soil	Trelogan		Prescot		Gateacre	
Sample	Initially	Average of three months	Initially	Average of three months	Initially	Average of three months
Unamended (pH = 6)	**100 (±13.3)**	**100 (±9.5)**	**3.0 (±0.4)**	**3.0 (±0.3)**	**5.0 (±1.0)**	**5.0 (±0.4)**
Unamended (pH = *)	**90 (±13.0)**	**90 (±7.20)**	**2.5 (±0.5)**	**2.4 (±0.6)**	**4.8 (±0.8)**	**5.0 (±0.5)**
P 0.5%	87 (±6.00)	78 (±7.00)	2.4 (±0.8)	2.0 (±0.3)	4.8 (±0.5)	4.0 (±0.4)
P 1%	85 (±5.00)	70 (±7.00)	2.3 (±0.3)	1.5 (±0.3)	4.6 (±0.7)	3.6 (±0.3)
P 5%	84 (±8.00)	54 (±5.00)	2.0 (±0.7)	1.4 (±0.3)	4.5 (±0.5)	3.2 (±0.5)
A 0.5%	86 (±6.00)	75 (±6.00)	2.5 (±0.2)	2.0 (±0.3)	4.8 (±0.4)	4.0 (±0.4)
A 1%	85 (±4.40)	62 (±6.20)	2.4 (±0.4)	1.6 (±0.2)	4.7 (±0.6)	3.4 (±0.6)
A 5%	86 (±5.30)	55 (±6.00)	2.3 (±0.3)	1.3 (±0.3)	4.6 (±0.4)	3.0 (±0.5)
Y 0.5%	90 (±8.50)	83 (±7.70)	2.6 (±0.4)	2.0 (±0.3)	5.0 (±2.0)	4.6 (±0.3)
Y 1%	87 (±7.00)	80 (±4.70)	2.5 (±0.5)	2.0 (±0.3)	4.8 (±0.7)	4.0 (±0.4)
Y 5%	85 (±11.0)	62 (±3.40)	2.4 (±0.3)	1.5 (±0.1)	4.4 (±1.2)	3.5 (±0.3)
+ 1% CaCO3	-	-	2.6 (±0.5)	2.5 (±0.4)	4.6 (±0.5)	4.4 (±0.3)

Note: Figures in brackets represent standard deviation; 6 = Initial pH (7.4 for Trelogan; 3.6 for Prescot; 4.6 for Gateacre);

* = Adjusted pH (10.0 for Trelogan; 6.5 for Prescot; 6.5 for Gateacre)

stable, and also that the zeolites did not decompose in the soils over the period of time analysed.

The bioavailable fraction of metals of the soil-zeolite mixture decreased with increasing the zeolite concentration and the changes in metal bioavailability were more significant in the soil system with the highest zeolite enrichment (5%).The trend of this relation was observed in all soil samples of the three types of soils under review.

The metal concentrations expressed as zeolite treatment : untreated control ratios were statistically analysed following the same procedure as described in Section 7.5. No significant differences were noted in the pH of the soils after three months incubation with zeolites, compared to the initial values. By adding the synthetic zeolites to the soils, the pH values increased, but they remained constant in time. For the pH of the leachates, Tukey tests reveal significant contrasts for all soils; for Trelogan soils, zeolite 4A at 0.5% versus zeolites P and Y at the same concentration, and also versus 1% concentration gave significant differences ($P = 0.002$); for Prescot soil, the only significant contrast ($P = 0.009$) was for 1% zeolite P versus Y, whereas for Gateacre soil, significant differences ($P = 0.000$) were noted for untreated control versus zeolite P in all concentrations and also versus 5% zeolites Y and 4A.

Highly significant contrasts ($P = 0.000$) were found with respect to the heavy metal concentrations in the leachates as a result of zeolite addition for all soil types. For Trelogan soil, the contrasts were between untreated control versus zeolites P and 4A at all concentrations and control versus zeolite Y at 5%. For Prescot and Gateacre soils, the contrasts were between untreated control versus zeolite P at all concentrations, zeolite 4A at 1% and 5% and zeolite Y at 5%.

The concentration of heavy metals in the soil solution indicate most accurately the rate of influx of metals from the soil into the plants. Changes in the concentrations of uncomplexed heavy metals with time will give the best correlations with changes in plant uptake of these metals over time, supporting the hypothesis that plants mainly absorb the free metal ion from the soil solution.

The decrease of the heavy metal content in the soil solution was the result of the addition of synthetic zeolites and a combination of both the zeolites ability to increase soil pH as well as the ion-exchange processes they caused in the soil, indicating that synthetic zeolites are effective treatments for immobilising heavy metals from contaminated soils.

Results of this study indicate that under conditions of reasonable zeolite application rates in metal-polluted soils, heavy metals remain unavailable in zeolite-amended soils for at least three months after zeolite application.

The efficiency of lime in reducing the bioavailable fraction of metals was approximately 2.5 times lower compared to that of synthetic zeolites. By increasing the pH of the soils, the amount of metals in the leachates decreased by 14% for copper, 25% for cadmium, 11% for zinc and 17% for lead after adding calcium carbonate to Prescot soil, whereas in Gateacre soil, the reductions were 22% for copper, 23.5% for cadmium, 10.3% for zinc and only 6% for lead.

In Prescot soil, the bioavailable fraction of copper decreased by 50% after adding zeolite P to this soil; zeolite 4A decreased the copper concentration by 46%, and zeolite Y by 44%(Table 6.3.). In Gateacre soil, zeolites P and 4A decreased the copper amount by 50%, whereas zeolite Y had a lower efficiency of only 43%. A 57% reduction in the bioavailable fraction of cadmium was noticed after adding 5% zeolites P and 4A to Prescot soil and a 43% decrease with zeolite Y. In Gateacre soil, zeolite 4A decreased cadmium availability by 58%, zeolite P by 50% and zeolite Y by 42% (Table 6.4.). The highest reductions were noticed for zinc in Prescot soil, that is 62% for zeolite P, 58% for zeolite 4A and 54% for zeolite Y. Zinc concentrations also decreased in Gateacre soil after the addition of synthetic zeolites: 53% for zeolite P, 56% for zeolite 4A and 42% for zeolite Y (Table 6.5.). The lowest metal decrease was noticed for lead: 42% for zeolite P, 46% for zeolite 4A, 37.5% for zeolite Y in Prescot soil, and 36% for zeolite P, 40% for zeolite 4A and 30% for zeolite Y in Gateacre soil (Table 6.6.).

After adding synthetic zeolites to the contaminated soils, the bioavailable fraction of metals decreased by over 40% for zeolites P and 4A and by 32% for zeolite Y in Trelogan soil, by more than 52% for zeolites P and 4A and by 45% for zeolite Y in Prescot soil, and by 47% for zeolite P, 51% for zeolite 4A and only by 39% for zeolite Y for Gateacre soil. This is compared to 7% copper, 16.7% cadmium, 11.5% zinc and 4% lead for lime addition to Prescot soil and 14.3% copper, 23% cadmium, 14% zinc and 4.3% lead with lime addition to Gateacre soil (Tables 6.3 - 6.6.).

Overall, the highest efficiency in decreasing the bioavailable fraction of metals in all soils was observed for zeolite P for copper and zinc. For cadmium, zeolites P and 4A had equal efficiency. For lead, only in Prescot soil, the efficiency of zeolite 4A was higher than for zeolite P. Zeolite Y had the lowest efficiency in all soils, due to its lower exchange capacity, with lime having the lowest effect for metal immobilisation.

6.4. Conclusions

For the range of soils studied, the amount of bioavailable metals decreased with an increase in zeolite concentration. All the above results were calculated as an average over three months from the results presented in Tables 6.3. - 6.6.

The results of the soil solution experiments are extremely important, as they show the ability of zeolites to retain the heavy metals already immobilised in their cages over a longer period of time. These results are also in agreement with those obtained by constructing the ion-exchange isotherms and by the sequential extraction experiments.

In addition, the superior efficiency of synthetic zeolites over lime as soil amendments, was demonstrated. The decrease in the bioavailable fraction of metals which was encountered by the column experiments has ecological significance, because the chemical composition of the soil solution reflects the demand of soil biological processes and, most important, as uptake of ions by plants occurs from the solution phase. Leaching experiments may provide information about the transfer of heavy metals from the solid to the liquid phase of the soil and the effects to be expected over a longer period of time, while sequential extraction techniques only reflect the situation at a given moment (Kiekens and Cottenie, 1982).

The experiments described in the present chapter were an attempt to gain a greater understanding of the action of the synthetic zeolites in a zeolite-soil system and, most important, constitutes one of the first column tests carried out with zeolite-amended soils.

Only few percolation studies, mainly prognostic or semi-field simulations have been carried out with natural clinoptilolite and with berengite and phillipsite, some of them with conflicting results: Wessolek and Fahrenhorst (1994) carried out prognostic simulations of cadmium and zinc transport in the soil profile of a sewage disposal site. With 8% berengite in the soil, the zinc concentration in the soil solution was reduced by a factor of 5, whereas cadmium was only reduced by a factor of 2-3. Semi-field percolation simulations under natural rainfall conditions of a waste dump substratum amended with 5% berengite were also carried out by Vangronsveld *et al.* (1995, ab). The zinc and cadmium concentrations of the passing percolate was decreased by more than 50%, as compared to the control. But the effect of berengite application to the surface substratum was somewhat conflicting: as in other similar cases (Vangronsveld *et al.*, 1994) it added to the success of revegetation and decreased the metal concentration in

the percolate, but on the other hand doubled the volume of percolated water. Kralova *et al* (1994) used phillipsite and natural clinoptilolite in model soil experiments where the zeolite enrichment changed from 1, 2 to 3%. The addition of synthetic phillipsite to soil samples increased the water holding capacity by 3 to 30% and cation exchange capacity by 10 to 50%. Field experiments were carried out with 0.25% phillipsite enrichment; the fields were fertilised with superphosphate (40 kg P to 1 ha) and potassium salts (60 kg K to 1 ha). The improvement of cation exchange capacity of the soil in field experiments was not observed, due to the low (0.25%) zeolite-enrichment used.

The laboratory testing procedures used in this study were designed to approach actual field conditions so more reliable predictions of pollutant fate and immobilisation effectiveness could be made. The column tests are the final stage of laboratory testwork and results from these and other work have lead to the choice of the most suitable synthetic zeolite for use in the field.

The previous chapters concentrated on the action of synthetic zeolites upon the first link of the food chain, and namely on their effect upon polluted soils. The following chapters will focus on the effect of synthetic zeolites on the second link of the food chain, namely the plants.

CHAPTER 7.
EFFECT OF ZEOLITE AMENDMENT ON PLANT GROWTH IN METAL CONTAMINATED SOILS.

7.1. Introduction

In many ways living plants can be compared to solar driven pumps which can extract and concentrate certain elements from their environment. All plants have the ability to accumulate, from soil and water, those heavy metals which are essential for their growth and development. These metals include iron, manganese, zinc, copper, magnesium, molybdenum, and possibly nickel. Certain plants also have the ability to accumulate heavy metals which have no known biological function, including cadmium, chromium, lead, cobalt, silver, selenium and mercury (Raskin *et al.*, 1994). Excessive accumulation of these heavy metals can be toxic to most plants.

Baker (1981) classified plants as excluder, indicator or accumulator plants, according to their ability to absorb heavy metals. Indicator plants tend to take up metals in amounts directly related to their availability in soil; excluders take up only small amounts, until a threshold value is exceeded, and uptake suddenly increases exponentially; and accumulator plants can bioconcentrate large amounts of metal from soils with little contamination.

At the concentrations generally present in soil solutions, the absorption of metals by plant roots is controlled by metabolic processes within the root. The proximity, extent, and pattern of contact between soil and root are important factors in the absorption of ions from soils and are especially important for the absorption of those metals which are tightly bonded to the soil colloids. The interaction of plant and soil may also change the composition of the soil solution at the root surface in ways which influence the absorption of metals from the solution.

The plant root modifies the soil environment in its immediate vicinity both by excreting chemically active substances and by absorbing water and ions. Mucilaginous material, possibly pectin, coats the surface of young roots and extends some distance into the soil. This and other excretions, such as organic acids, amino acids, HCO_3^-, and H^+ ions, may affect the release of metals from soil colloids. Excretions may also stimulate the activity of microorganisms, which may in turn affect the bioavailability of metals both by competing with the plant root for their absorption and by affecting their release from soil colloids (Lepp, 1981).

In soil-plant systems there are five main pathways of toxic metal transport and transformation: (i) *absorption*, via root uptake and possibly via stomata and foliar absorption; (ii) *redistribution*, via xylem and phloem transport; (iii) *metabolism*, in biosynthesis; (iv) *excretion*, in litterfall and root decay; (v) *recycling*, via decomposition and mobilisation processes (Ross, 1994).

The production of agricultural plants can be depressed by the effect of heavy metals. Crops may be negatively affected in regions where higher concentrations of these metals are present in the soil or water. Research of the mechanism of the effect of these elements on growth, development and metabolism of plants can provide valuable information to adopt improving measures. In addition, permanent protection of the environment is necessary to prevent the accumulation of heavy metals in the biosphere (Stiborova *et al.*, 1986).

Growth conditions are further determinants of the extent of metal uptake by plants. Field experiments have shown that heavy metal contents in plants vary from season to season due to differences in ambient or soil temperatures, and precipitation (Chang *et al.*, 1987) . Heavy metal concentrations in plants also change during physiological development. In general, young plants contain greater concentrations of nutrient and heavy metal ions than older, less metabolically active plants.

Crops differ in their ability to take up, accumulate and tolerate heavy metals. Different plant species take up different amounts of heavy metals, even when grown under identical conditions (e.g. Turner, 1973; Zhang *et al.*, 1991; Frossard, 1993). The mobility differs between metals, according to the type of soil and type and degree of contamination being considered. There is general agreement that cadmium and zinc are relatively more mobile in soils than elements such as copper and lead.

The possibilities of movement of various metals into plants are not equal. A plant is capable of retaining a large amount of lead in its roots and hence weakening the flow of excess ions into its tops. The slowest component of metal removal, reported for high lead concentrations (Dushenkov *et al.*, 1995), involves root-mediated precipitation from the soil solution in the form of insoluble lead phosphate. This precipitation probably involves a release of root exudates. In addition, cell walls of roots exposed to lead accumulate large amounts of insoluble lead carbonate, formed from respiratory carbon dioxide.

Copper tends to be very strongly bound to soil organic matter and is mainly non-toxic to plants. However, copper is accumulated in plant roots, where it hinders the uptake of essential nutrients and thus retards plant growth (Brams and Fiskell, 1971).

Cadmium and zinc show the greatest tendency to move along the soil-to-plant pathway (Alloway and Jackson, 1991). They move comparatively easily from the roots to the stems and can accumulate at high concentration in storage organs.

With regard to the toxicity of metals to plants, Davis and Carlton-Smith (1984) found that the relative toxicities to ryegrass, (*Lolium perenne)* of zinc, copper and nickel were 1.0:2.6:1.0 at pH 7. Wong and Bradshaw (1982) comparing the toxicity sequence of heavy metals for *Lolium perenne* (Cu > Ni > Mn > Pb > Cd > Zn > Hg > Cr > Fe) found them compatible with the order of stability of metal organic complexes. Burridge and Berrow (1984) found copper concentrations in ryegrass and clover decreased with time, but nickel and zinc remained highly available.

Early studies by Goldschmidt (1958) showed that the roots of perennial ryegrass had the ability to rapidly take up almost all lead from a lead nitrate solution. Lead bound in the roots was not released by exchange with calcium or barium ions, which precluded simple absorption or metabolic processes. Also, the transport of lead from the roots to the shoots did not exceed 29% of total uptake. Therefore, the roots of actively growing ryegrass provided a barrier which restricts the movement of lead to the above-ground parts of the plants, and so to animals or man (Jones *et al.*, 1973).

Jarvis *et al.* (1976) have found that cadmium uptake by the roots of ryegrass was considerably depressed by calcium, manganese and zinc by competing for exchange sites at the root surface.

The considerable mobility of cadmium and zinc in the soil-plant system represents a potential hazard to human health when levels of these two metals in soils are high (Jackson and Alloway, 1990). Less cadmium was accumulated in the shoots of grape vines grown on contaminated soils than to sunflower, maize, wheat or ryegrass and toxicity was more severe on acid soils than on those with a higher pH (Mohr, 1985). Studies with maize show that cadmium is bound by the cell-wall proteins of the apoplast or deposited in the vacuoles of root tissues. Rhizospheric interactions may also play a part, since root mucilages and soluble root exudates from maize have been shown to posses cadmium-binding properties. In maize, copper and zinc contents were higher in the leaves than in the seeds (Morel *et al.*, 1986; Mench *et al.*, 1987).

The rate of metal accumulation can be rapid. Salt *et al.* (1995) found that within 24 hours, roots of sunflower (*Helianthus annuus L.*) were able to dramatically reduce the levels of chromium (VI), manganese, cadmium, lead, zinc and copper in water, such that metal concentrations were reduced to close or below the regulatory discharge limits.

Metalliferous substrates tend to be heterogeneous, varying both in terms of background edaphic characteristics and heavy metal content (Smith & Bradshaw, 1972). Plant survival and tolerance are more difficult to predict when the metal species and concentration are variable because the severity of contamination differs according to the source of metal pollution, site topography, soil structure, fertility and vegetation. The most important background edaphic factors which directly affect heavy metal availability are pH, organic matter content, exchangeable cations and nutrient status, as well as the presence of other pollutants (Livens, 1991; Alloway, 1995).

The work described here investigates the survival and biomass production of four selected test plants grown on contaminated substrates from three different sites. pH, moisture content, organic matter content, exchangeable cations, water- and nitric acid-extractable concentrations of copper, cadmium, zinc and lead were measured at the beginning of the experiment. The substrates were then amended with three synthetic zeolites (P, 4A and Y) at concentrations of 0.50%, 1.00% and 5.00% (w/w) dry wt. together with untreated soil samples as controls. This study aims to understand how previous ion-exchange studies translate into a real-world situation by investigating the biological response of plants tested in contaminated substrates amended with synthetic zeolites.

Sunflower (*Helianthus annuus*), maize (*Zea mays*), ryegrass (*Lolium perenne*) and willows (*Salix viminalis*) were grown in the polluted soils with different zeolite amendments as described above. The plant species were selected on the basis of their inherent capacity for metal uptake and their use for food, fodder or biomass purposes.

Perennial ryegrass was grown because of its importance in the food chain from soils through animal products to man. More than 40% of permanent grassland in England and Wales contains about 1/3 perennial ryegrass and it is seldom absent from enclosed pastures (Hopkins, 1990) because it survives well under conditions of high nitrogen input implemented by most farmers. Sunflower and maize are plants with a high industrial importance, and studying the heavy metal bioaccumulation in their parts is imperative. In addition to their agricultural importance, these plants were selected because previous studies (Jones *et al.*, 1973; Salt *et al.*, 1995; Mench *et al.*, 1987) have demonstrated their intrinsic ability to accumulate heavy metals from solution.

Willow, *Salix viminalis* was chosen as a test plant because it grows well in elevated copper situations (Punshon *et al.*, 1994) and is tolerant to extremely high levels of lead (17000 $\mu g\ g^{-1}$) (Eltrop *et al.*, 1991). The potential use of fast growing clones of species of *Salix* in short rotation forestry as a biomass crop is well established (Sommerville, 1992).

Some willow genotypes are able to survive on a wide range of metal-contaminated soils, to allow revegetation, and may potentially be used to accumulate metals in tissues which could then be harvested to remediate or polish contaminated soils.

7.2. Experimental

7.2.1. Material and Methods

Plant trials with mono- and dicotyledonous plants were carried out in order to estimate the ability of zeolites to improve plant growth and reduce the concentration of heavy metals in plants. The effect of synthetic zeolites on the pool of plant-available toxic metals was investigated by adding zeolites P, 4A and Y at controlled levels to each of the soils described (0.50%, 1.00% and 5.00% w/w, d.w.). Untreated soil samples served as controls.

The pots were filled with the contaminated soils containing appropriate amendments (4 kg, dry weight basis) and fertilised with John Innes Base Fertiliser (0.16 g dm^{-3} soil). For each greenhouse experiment, the amendments were thoroughly mixed with the soils and left to equilibrate for 7 days to allow the reaction of the zeolite with the soil and fertiliser to stabilise.

Sunflower *(Helianthus anuus, var.* Rodeo*)* and maize *(Zea mays, var.* Fronica*)* were planted into pots (4 seeds/pot) and after germination, the plants were thinned to 2 plants per pot. The plants were harvested after three months.

Perennial ryegrass *(Lolium perenne, var.* Elka*)* was sown (1.5 g/kg of dried soil) in plastic trays each containing 1 kg of amended soil and the resulting growth was harvested after six weeks.

One year old willow rods of *Salix viminalis* (Clone 379) were collected from the National Willow Collection in Ness Botanic Gardens in May 1994. The rods were prepared for planting by removing all leaf material and cutting down to 10 cm lengths. The cuttings were maintained in 2.5 litre buckets containing 1 litre distilled water for 5 days prior to planting, to prevent drying out. Cuttings were planted with approximately 8 cm beneath the soil, with 4 cuttings per pot. After shooting, the cuttings were thinned to 2 per pot. The reference uncontaminated soil chosen for this experiment was John Innes No.1 potting compost, with a pH of 5.6.

The pots were placed randomly in an enclosure on the roof of the University Buildings and watered daily during the summer months, with any weeds removed by hand. Cuttings were allowed to grow for one year and some foliage was harvested in May 1995.

7.2.2. Harvesting and analysis of plant material

Following the completion of the treatment schedule, the plant material was removed from the pots and care was taken not to damage any fine roots, which were cleaned thoroughly with distilled water to remove soil. Plant material was separated into leaves (plus petioles) and roots, the latter being washed with distilled water to remove any adherent soil particles. All plant samples were dried at 60°C for 72 hours, weighed and ground in a mechanical sample grinder. The resulting biomass (fresh and dry) was weighed to give a yield figure (g dry wt.) of material produced per pot. The plant material was also analysed in triplicate for concentrations of copper, cadmium, zinc and lead. Estimates of metal accumulation were calculated (in μg) from yield and metal analysis data, giving approximate figures for metal content in individual plant compartments and total uptake, expressed as mg of metal per pot dry weight.

7.3. Microwave digestion of plant material

The methods described are optimised conditions for the plant material analysed in this study using manufacturers recommendations as a base line. The samples were separated into leaves and roots for ease of digest.

7.3.1. Leaves

A MDS-81D Microwave digestion system was used as described in section 4.2.1.2. Approximately 0.5 g of finely ground sample was accurately weighed into each Teflon digestion vessel after which HNO_3 (10 cm^3; conc., A.R.) was added in a fume cupboard. The vessels were allowed to stand for 30 minutes and a further aliquot of HNO_3 (5 cm^3; conc., A.R.) was added to each sample. A safety valve and cap were placed on each vessel and tightened using the capping station. Each vessel was numbered, a venting tube attached and placed on the turntable with fan and turntable activated. The oven was programmed for 4 minutes at 100% power (step 1) and 8 minutes at 50% power (step 2). Samples were

removed and checked for any venting or loss of material, then allowed to cool at room temperature. The vessels were then manually vented and digested samples were transferred to 25 cm^3 volumetric flasks. Using this programme, the samples were digested until completely clear, and the filtration stage was omitted. The samples were diluted to volume using distilled water and analysed using atomic absorption spectroscopy. Triplicate samples were prepared with nitric acid blanks.

7.3.2. Roots

0.5 g of dried, ground (1 mm) material was prepared for digestion as described in the previous section. For 12 vessels the program was set at 20 minutes at 35% power after which the samples were removed and allowed to cool to room temperature. Vessels were tightened using the capping station and replaced. The oven was programmed for a further 10 minutes at 65% power. The samples were allowed to cool to room temperature and the clear solutions were transferred to 25 cm^3 volumetric flasks and diluted as above. Again, triplicate samples and blanks were digested.

Between digests the Teflon vessels, caps, safety valves and venting tubes were decontaminated by washing with tap water and Decon detergent followed by concentrated nitric acid and distilled water after which they were dried at 60°C. Vessels were additionally decontaminated by microwaving at full power for 20 minutes with HNO_3 (15 cm^3; conc., A.R.) between experiments.

7.4. Growth observations

The need for watering and randomisation of the pots ensured that regular observations of the pots were made during growth. A photographic record was taken at various stages for all treatments.

7.5. Statistical analysis

A two-way analysis of variance (ANOVA) was used to test for differences in mean metal concentration between zeolite type/concentration and between metal types, as well as the interaction between these two factors. Because of the large data set, emphasis was placed

on graphical representation of data and grouping of factors used in analyses of variance to facilitate interpretation.

7.6. Results of Plant Trials

7.6.1. Plant Growth

Plant growth trials in a range of polluted soils have indicated that a beneficial and positive effect on growth of all test species studied was observed by the addition of synthetic zeolites to the contaminated soils (Tables 7.1. - 7.4.).

Growth damage was found in the test plants grown on all the unamended soils containing high levels of metals, compared to the plants grown in amended soils. Yields on the amended soils were not significantly different from the compost control. Dry matter yields of test plants grown in the unamended and amended polluted soils are presented in Tables 7.1. - 7.4.

Visual analysis of the most relevant effects of synthetic zeolites P, 4A and Y on plant growth are presented in the following plates: 7.1. a.-d.(ryegrass); 7.2. a.-d.(sunflower); 7.3. a.-d.(maize) and 7.4. a.-d. (willow).

In these experiments, unamended soils containing high levels of heavy metals restricted the growth and reduced the dry matter yield of the test plants. Plants grown in the unamended polluted soils (particularly in Trelogan soil) developed toxicity symptoms in the leaves as observed in pot studies. In the untreated polluted soils, the plants showed typical symptoms of heavy metal pollution, such as necrosis and chlorotic leaves.

The high levels of metals at Trelogan site, both total and water soluble, together with the low organic matter content and poor soil structure had a cumulative effect on the performance of the plants.

None of the sunflower seeds sown in Trelogan soil germinated, due to the extremely high levels of heavy metals in this soil (Plate 7.2.a.). Maize and ryegrass survived in Trelogan mine spoil but produced a very limited amount of biomass. Chlorosis and dessication was rapid for ryegrass (Plate 7.1.a.). Chlorosis was also observed in the stunted growth of the maize plants (Plate 7.3.a.). All willow growing in Trelogan soil presented typical symptoms of heavy metal pollution, such as leaf necrosis, chlorotic leaves, serious loss of leaves and showed distinctive reddening of new stem material (Plate 7.4.a.). This is also a characteristic

Table 7.1. Sunflower dry wt.(g) grown in contaminated soils amended with zeolites (2 plants/pot).

Soil	Prescot							
Part	Roots				Foliage			
Conc.	No zeolite	0.50%	1.00%	5.00%	No zeolite	0.50%	1.00%	5.00%
Zeo P	N.G.	0.88	1.41	N.G.	N.G.	2.28	2.62	N.G.
Zeo 4A	N.G.	1.72	0.61	N.G.	N.G.	6	2.05	N.G.
Zeo Y	N.G.	1.3	0.71	0.32	N.G.	3.4	2	1.97
Soil	Gateacre							
Part	Roots				Foliage			
Conc.	No zeolite	0.50%	1.00%	5.00%	No zeolite	0.50%	1.00%	5.00%
Zeo P	2.05	1.7	2.65	N.G.	8.76	6.4	9.73	1.06
Zeo 4A	2.05	3.14	0.33	0.54	8.76	11	2.41	2
Zeo Y	2.05	1.22	1	2.47	8.76	6	6.66	7.66

Note: N.G. = No plant growth

* = No plant growth on amended and unamended Trelogan soil (see text).

Table 7.2. Maize dry wt.(g) grown in contaminated soils amended with zeolites (2 plants/pot).

Soil	Prescot							
Part	Roots				Foliage			
Conc.	No zeolite	0.50%	1.00%	5.00%	No zeolite	0.50%	1.00%	5.00%
Zeo P	0.17	1.87	2.68	N.G.	0.34	5.48	6.34	N.G.
Zeo 4A	0.17	2.72	4.03	N.G.	0.34	5.95	8	N.G.
Zeo Y	0.17	0.11	0.4	0.74	0.34	0.28	1.9	4.25
Soil	Gateacre							
Part	Roots				Foliage			
Conc.	No zeolite	0.50%	1.00%	5.00%	No zeolite	0.50%	1.00%	5.00%
Zeo P	4.37	2.7	0.39	0.35	13.23	8.44	1.81	0.32
Zeo 4A	4.37	4.5	4.05	N.G.	13.23	10.27	8.18	0.27
Zeo Y	4.37	1.68	6.91	0.53	13.23	5.66	9.72	4

Note: N.G. = No plant growth

* = No plant growth on amended and unamended Trelogan soil (see text).

Table 7.3. Ryegrass foliage dry wt.(g) grown in contaminated soils amended with zeolites (2 plants/pot).

Soil	Conc.	Zeo P	Zeo 4A	Zeo Y
Prescot	No zeolite	1.12	1.12	1.12
Prescot	0.50%	3.90	5.65	3.23
Prescot	1.00%	6.42	6.65	6.40
Prescot	5.00%	0.77	N.G.	5.60
Gateacre	No zeolite	10.7	10.7	10.7
Gateacre	0.50%	16.3	18.1	13.5
Gateacre	1.00%	14.8	17.5	16.9
Gateacre	5.00%	0.64	6.50	18.1

Note: N.G. = No plant growth

* = No plant growth on amended and unamended Trelogan soil (see text).

Table 7.4. Willow dry wt.(g) grown in contaminated soils amended with zeolites (2 plants/pot).

Soil	Prescot							
Part	Shoots				Leaves			
Conc.	No zeolite	0.50%	1.00%	5.00%	No zeolite	0.50%	1.00%	5.00%
Zeo P	1.24	1.34	1.38	N.G.	2.1	3	3.1	N.G.
Zeo 4A	1.24	0.86	1	N.G.	2.1	2.74	2.58	N.G.
Zeo Y	1.24	0.45	1.09	0.38	2.1	1.16	2.49	0.63
Soil	Gateacre							
Part	Shoots				Leaves			
Conc.	No zeolite	0.50%	1.00%	5.00%	No zeolite	0.50%	1.00%	5.00%
Zeo P	1.14	1.21	1.71	N.G.	2.47	3.36	2.80	N.G.
Zeo 4A	1.14	1.28	1.62	N.G.	2.47	3.7	2.61	N.G.
Zeo Y	1.14	N.G.	N.G.	1.6	2.47	N.G.	N.G.	3.9

Note: N.G. = No plant growth

* = No plant growth on amended and unamended Trelogan soil (see text).

Plate 7.1. a. Effect of zeolite Y on ryegrass treatments.

Left to right: Control; Trelogan; Trelogan + 0.5% Zeolite Y;
Trelogan + 1% Zeolite Y; Trelogan + 5% Zeolite Y.

Plate 7.1. b. Effect of zeolite 4A on ryegrass treatments.

Left to right: Control; Gateacre; Gateacre + 0.5% Zeolite 4A;
Gateacre + 1% Zeolite 4A; Gateacre + 5% Zeolite 4A.

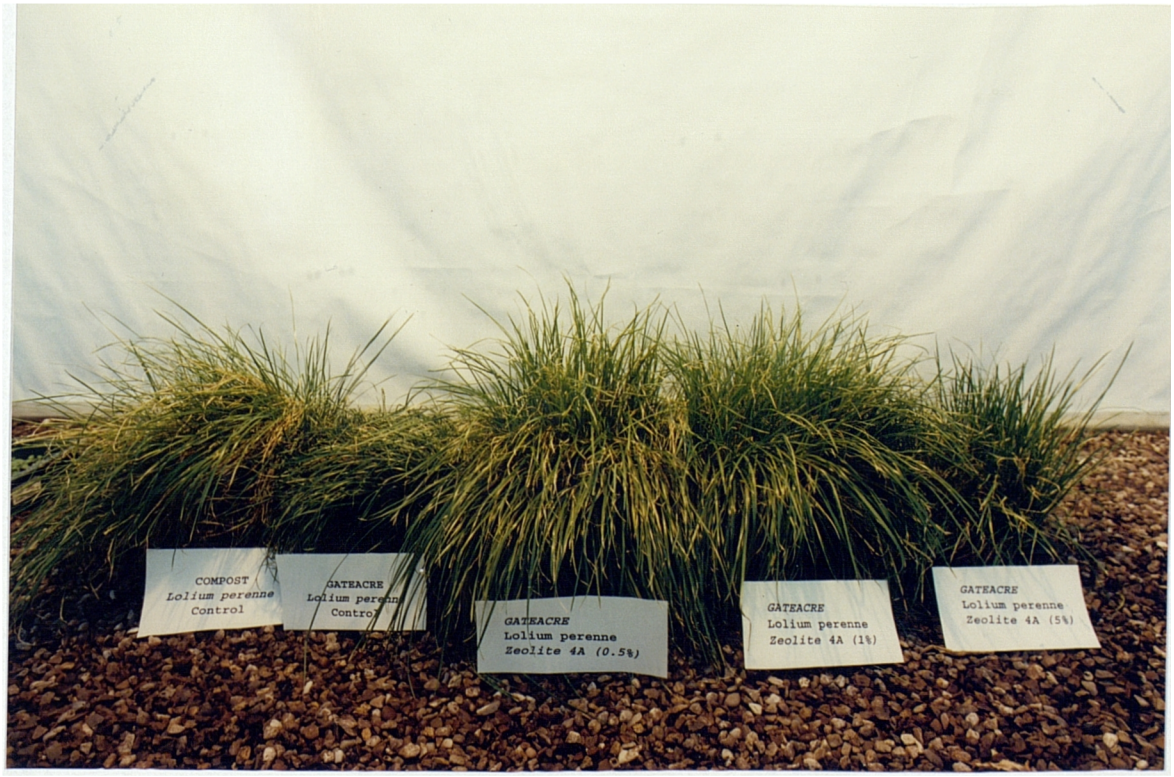


Plate 7.1. c. Effect of zeolite 4A on ryegrass treatments.

Left to right: Control; Prescot; Prescot + 0.5% Zeolite 4A;
Prescot + 1% Zeolite 4A; Prescot + 5% Zeolite 4A.

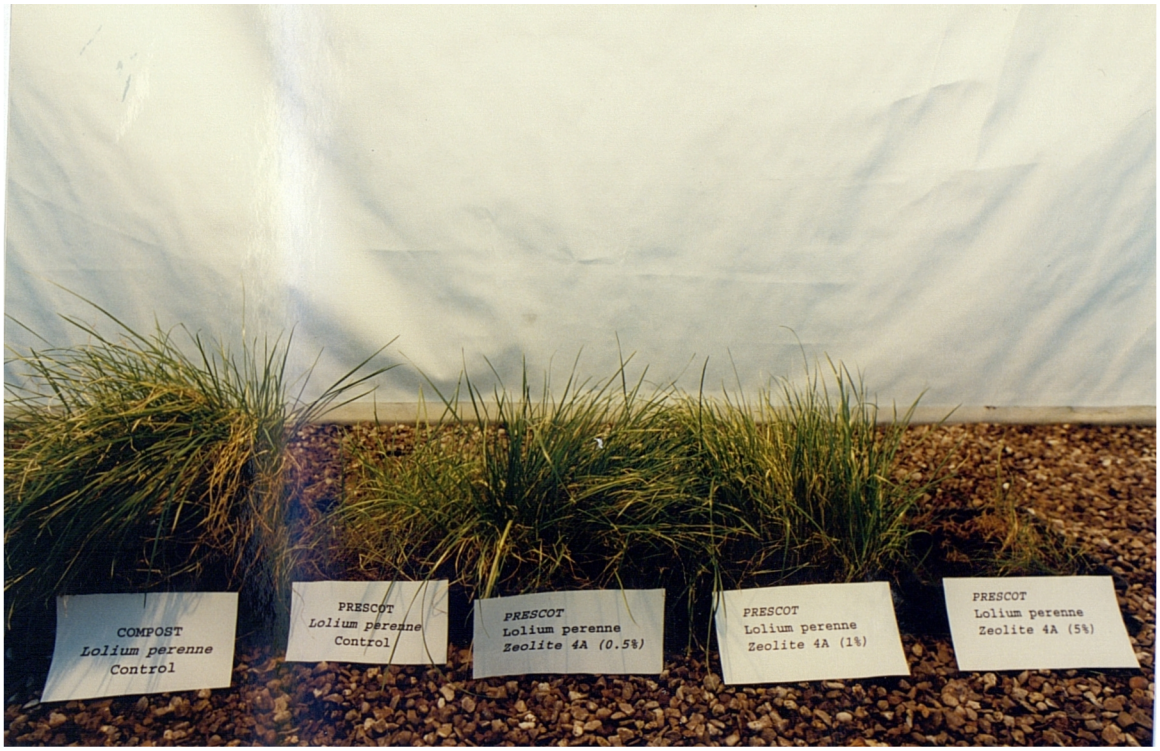


Plate 7.1. d. Effect of zeolites P, 4A and Y on ryegrass treatments.

Left to right: Control; Prescot; Prescot + 5% Zeolite P;
Prescot + 5% Zeolite 4A; Prescot + 5% Zeolite Y.

Plate 7.2. a. Effect of zeolite Y on sunflower treatments.

Left to right: Control; Trelogan; Trelogan + 0.5% Zeolite Y;
Trelogan + 1% Zeolite Y; Trelogan + 5% Zeolite Y.

Plate 7.2. b. Effect of zeolites P, 4A and Y on sunflower treatments.

Left to right: Control; Prescot; Prescot + 0.5% Zeolite P;
Prescot + 0.5% Zeolite 4A; Prescot + 0.5% Zeolite Y.

Plate 7.2. c. Effect of zeolite P on sunflower treatments.

Left to right: Control; Gateacre; Gateacre + 0.5% Zeolite P;
Gateacre + 1% Zeolite P; Gateacre + 5% Zeolite P.

Plate 7.2. d. Effect of zeolites P, 4A and Y on sunflower treatments.

Left to right: Control; Gateacre; Gateacre + 5% Zeolite P;
Gateacre + 5% Zeolite 4A; Gateacre + 5% Zeolite Y.

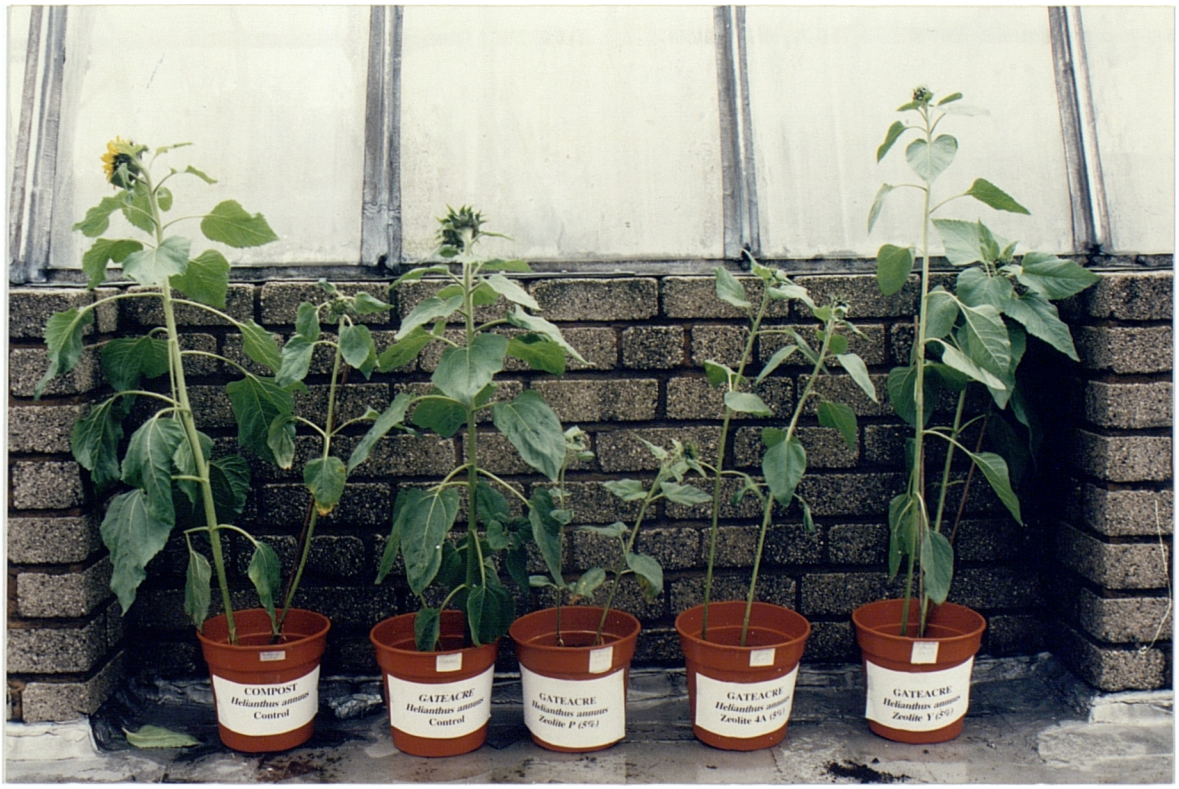


Plate 7.3. a. Effect of zeolites P, 4A and Y on maize treatments.

Left to right: Control; Trelogan; Trelogan + 1% Zeolite P;
Trelogan + 1% Zeolite 4A; Trelogan + 1% Zeolite Y.

Plate 7.3. b. Effect of zeolites P, 4A and Y on maize treatments.

Left to right: Control; Prescot; Prescot + 1% Zeolite P;
Prescot + 1% Zeolite 4A; Prescot + 1% Zeolite Y.

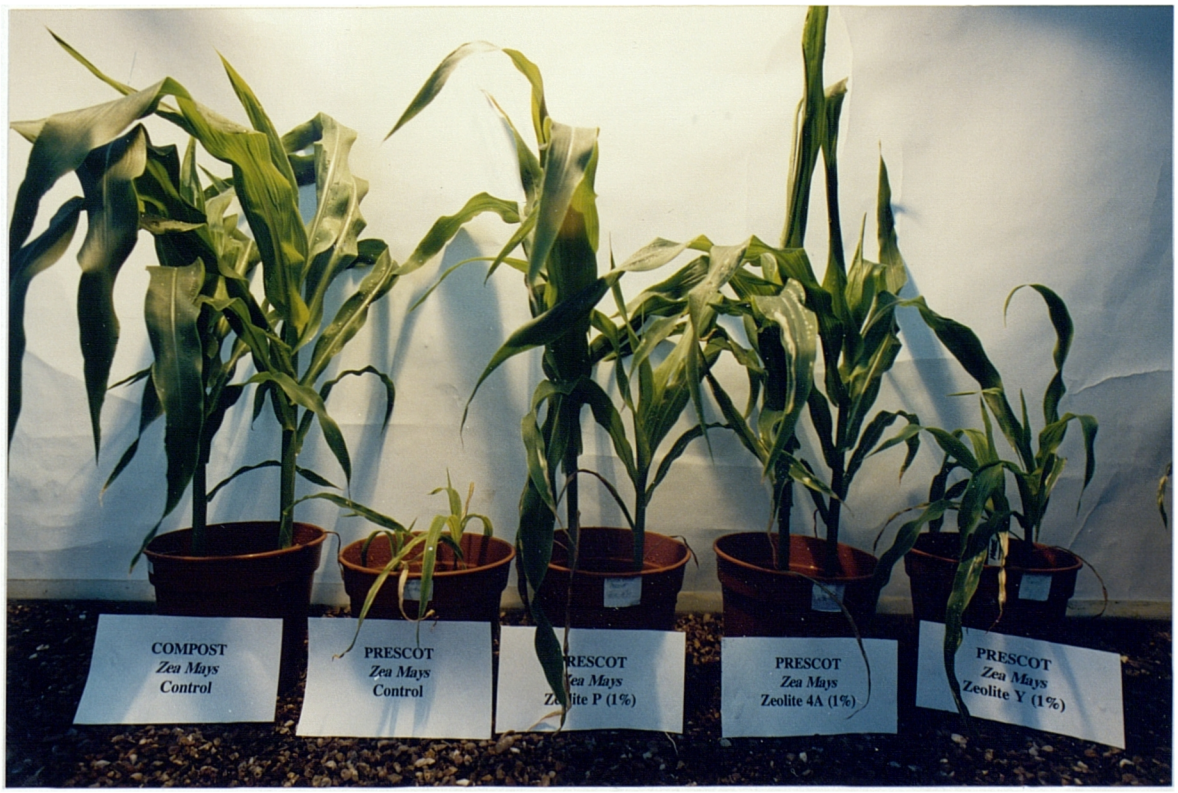


Plate 7.3. c. Effect of zeolite 4A on maize treatments.

Left to right: Control; Gateacre; Gateacre + 0.5% Zeolite 4A;
Gateacre + 1% Zeolite 4A; Gateacre + 5% Zeolite 4A.

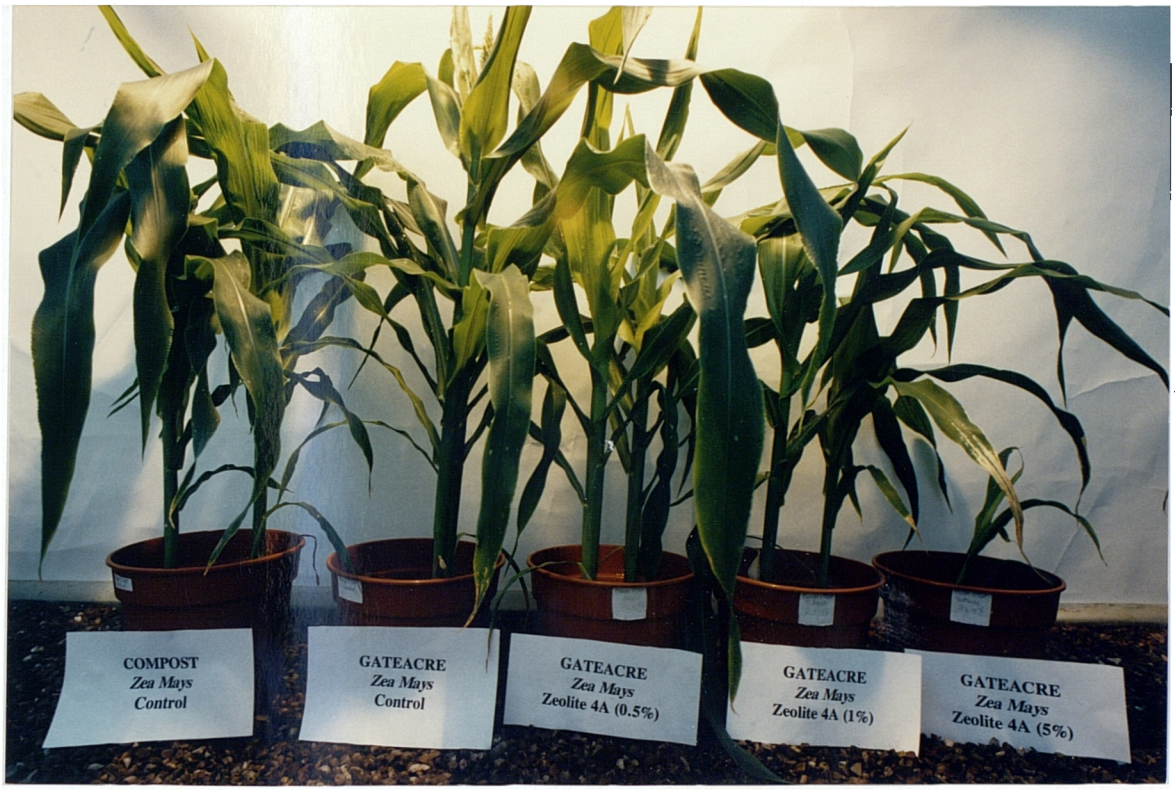


Plate 7.3. d. Effect of zeolites P, 4A and Y on maize treatments.

Left to right: Control; Gateacre; Gateacre + 5% Zeolite P;
Gateacre + 5% Zeolite 4A; Gateacre + 5% Zeolite Y.

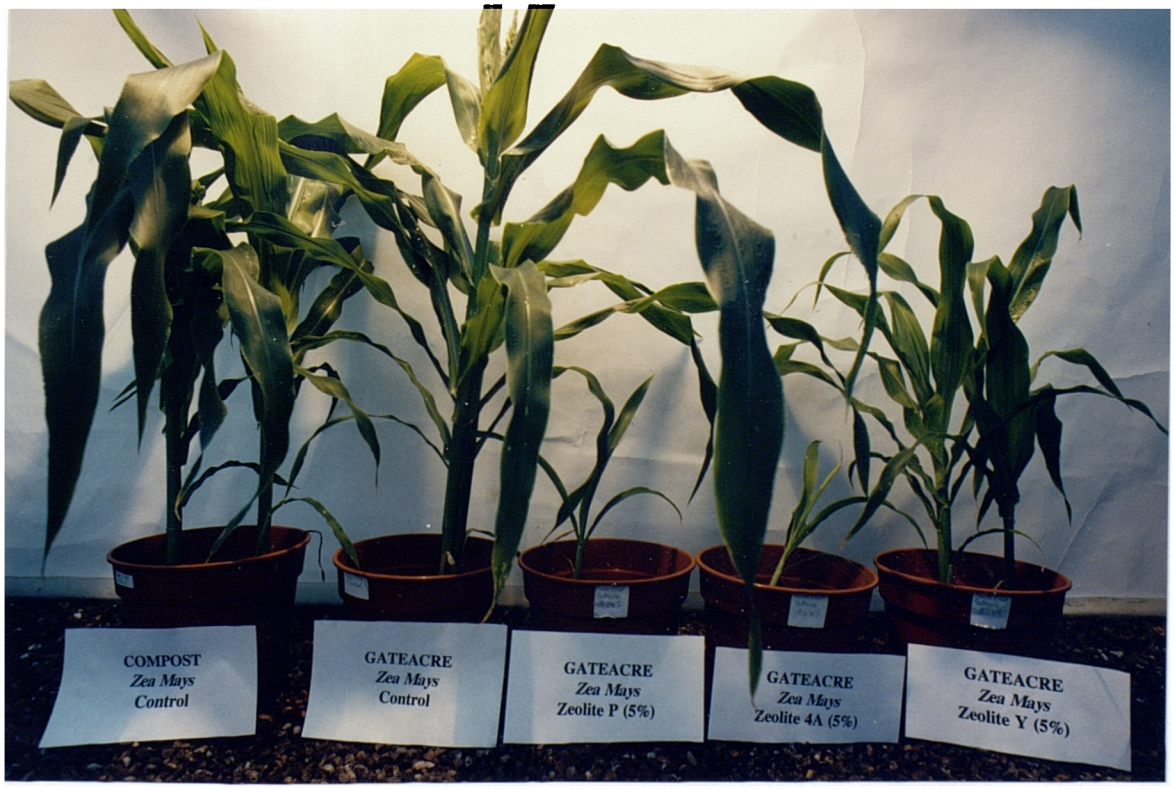


Plate 7.4. a. Effect of zeolites P, 4A and Y on willow treatments.

Left to right: Control; Trelogan; Trelogan + 0.5% Zeolite P;
Trelogan + 0.5% Zeolite 4A; Trelogan + 0.5% Zeolite Y.

Plate 7.4. b. Effect of zeolite 4A on willow treatments.

Left to right: Control; Prescot; Prescot + 0.5% Zeolite 4A;
Prescot + 1% Zeolite 4A; Prescot + 5% Zeolite 4A.

Plate 7.4. c. Effect of zeolites P, 4A and Y on willow treatments.

Left to right: Control; Gateacre; Gateacre + 0.5% Zeolite P;
Gateacre + 0.5% Zeolite 4A; Gateacre + 0.5% Zeolite Y.

Plate 7.4. d. Effect of zeolites P, 4A and Y on willow treatments.

Left to right: Control; Gateacre; Gateacre + 5% Zeolite P;
Gateacre + 5% Zeolite 4A; Gateacre + 5% Zeolite Y.

of native Trelogan willows. In unamended Trelogan soil, the willow shoots did not survive. *Salix viminalis* failed to produce enough material in Trelogan soil for metal analysis.

Despite the addition of zeolites to this soil, there was no observable improvement in the quality of the plants. This is due not only to the extremely high toxic metal levels (Trelogan soil contains extremely high levels of lead and zinc (Table 4.4.), but also a lack of nutrients in the low organic content of the soil. The organic matter content of Trelogan soil was 3.5%. On the basis of the low value for this parameter, a rather low binding of metals to organic matter was presumed. Therefore, input of organic matter (compost) would improve the metal binding capacity of Trelogan soil. Viability was lowest in plants grown in Trelogan mine spoil.

The strong metal immobilisation capacity of zeolites in metal-contaminated soils was confirmed for Gateacre soil; the addition of zeolites P or 4A at 0.5% and 1% rates showed better growth response as compared to the unamended soil and resulted in a complete disappearance of visual symptoms of metal phytotoxicity (Plates 7.1.b., 7.2.c., 7.3.c. and 7.4.c.). For the sunflower plants, zeolite Y at 5% application rate showed a comparable growth to that of compost (Plate 7.2.d.). Zeolite P at 0.5% and 1% concentrations improved significantly the plant growth (Plate 7.2.c.). However, when the application of zeolites P and 4A was 5%, growth was significantly reduced in all the crops studied (Plates 7.1.b., 7.2.d. and 7.3.d.). Only small maize plants were observed in Gateacre soil amended with 5% zeolite P and 4A respectively (Plate 7.3.d.).

Ryegrass growth in Gateacre soil improved on addition of zeolites, but showed no apparent visual differences between zeolite treatments, except that zeolites 4A and P at 5% concentration had an adverse effect (Plate 7.1.b.).

Many of the successful willows grown in Gateacre soil recovered after initially showing severe signs of metal toxicity, by producing new shoots and buds (Plate 7.4.c.). Willows grew successfully on Prescot and Gateacre soils and showed comparable growth to plants grown in John Innes Compost (Plates 7.4.b., 7.4.c., 7.4.d.). Prescot soil is contaminated primarily with copper. The growth of ryegrass on Prescot soil amended with zeolite 4A at 0.5% or 1% was comparable to that of the compost control (Plate 7.1.c.). The 5% application rate for zeolites P and 4A proved far in excess, as ryegrass presented acute chlorosis and drying (Plate 7.1.d.). The willow plants were much greener in zeolite-amended Prescot soil than in the untreated soil (Plate 7.4.b.). In the unamended soil, sunflower seedlings developed severe leaf chlorosis within a week of sowing followed by necrosis and death (Plate 7.2.b.). The maize plants did survive in

unamended Prescot soil (Plate 7.3.b.), although they presented acute symptoms of heavy metal contamination, such as stunted growth and chlorosis. Maize is a monocotyledonous plant which is known to have a higher tolerance to heavy metal contamination than dicotyledonous plants such as sunflower. This is also confirmed by the fact that even in the highly heavy metal contaminated Trelogan soil, the maize seeds germinated, although the plants were very small and chlorotic (Plate 7.3.a.). These observations are in agreement with other studies. Stoilov and Popov (1984) reported an increase in maize yield (10%) and the acceleration of ripening when maize was grown in soil amended with clinoptilolite, a naturally occurring zeolite.

Addition of synthetic zeolites to Prescot soil increased the biomass and reduced the metal content of the maize plants studied. A similar pattern for the levels of zeolite amendment to that described for Gateacre soil was also observed in Prescot soil.

Plant growth is considerably improved after adding synthetic zeolites to the polluted soils. Each zeolite was effective to some extent, and in all cases an optimum rate of addition could be detected.

Zeolites P and 4A were most effective at low rates of application but became detrimental to plant growth at higher application rates (Plates 7.1.c., 7.2.c., 7.3.c. and 7.4.b.). Zeolite Y was most effective at the 5% treatment rate; higher quantities of zeolite Y are needed due to its lower exchange capacity (Plates 7.1.d., 7.2.d., 7.3.d. and 7.4.d.).

The poor plant growth (Plates 7.1.d., 7.3.d. and 7.4.d.) observed when zeolites 4A and P were used at the higher application rate (5.00%) can be attributed to the high concentration of zeolite reducing not only the metal content of the soil, but also reducing the availability of essential nutrients by the same ion exchange process.

The addition of an exchanger may reduce the heavy metal availability, but the exchanger also absorbs essential cations. This reduced cation availability can lead to nutrient deficiencies (Mohr, 1982).

The results showed that synthetic zeolite application is a feasible method for improving plant growing conditions on highly polluted soils.

7.6.2. Accumulation of heavy metals by test plants.

Metal analyses showed that all plants grown in unamended contaminated soils accumulated high concentrations of all metals, particularly cadmium, lead and zinc.

These high concentrations explain why the plants performed so poorly in unamended soils. The study of metal accumulation was very difficult in some cases because of lack of plant material for metal analysis. Although heavy metal uptake into the primary producer trophic level has decreased after zeolite applications, metals are still present at significant levels.

The monocotyledonous plants with fibrous root systems responded more strongly to heavy metal immobilisation by synthetic zeolites than did dicotyledonous plants with tap root systems. The C.E.C. of the dicotyledons is twice as high as that of monocotyledons, and the differences have been ascribed to the contents of pectic substances in the roots (Iwasaki *et al.*, 1990).

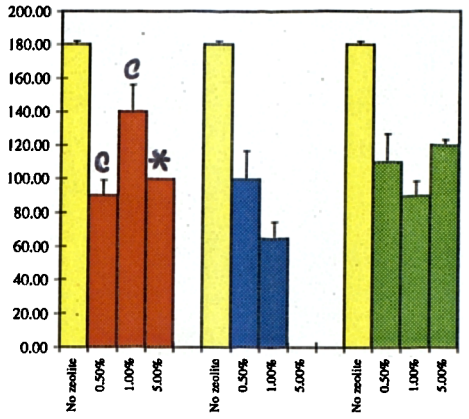
The use of synthetic zeolites as a treatment additive for the immobilisation of heavy metals has proved effective for Prescot and Gateacre soils. Plants grown in soils from both sites which had been amended with differing levels of each zeolite showed considerable reductions in the concentration of the contaminating metals (Fig. 7.1.-7.56.).

The total metal uptake by plants was calculated by multiplying the metal concentration with dry matter yields of the plant species for each pot. Therefore, the effects of synthetic zeolites on the total metal uptake depended upon their effects on both the metal concentrations and dry matter yields of the plant species. These data are presented in Appendix 4 (Tables 4.1.-4.7.).

General Observations

Adding 0.5% zeolite P to Prescot soil decreased the total metal uptake of sunflowers by up to 0.264 mg zinc/pot for roots and 0.41 mg copper/pot for foliage, values which are far below the total uptake for the sunflower plants grown in compost control (0.38 mg zinc/pot for roots and 0.87 mg copper/pot for foliage)(Appendix 4 - Table 4.2.). Zeolite P 0.5% decreased the total metal uptake of cadmium and also zinc for the sunflower plants grown on amended Gateacre soil. Cadmium uptake for sunflower roots was 0.031 mg cadmium/pot for unamended Gateacre soil in comparison to 0.017 mg cadmium/pot for zeolite P-amended soil. Cadmium availability for the sunflower foliage decreased by 67% in the presence of 0.5% zeolite P (0.079 mg cadmium/pot for unamended Gateacre soil and only 0.026 mg cadmium/pot for amended soil)(Appendix 4 - Table 4.3.) .

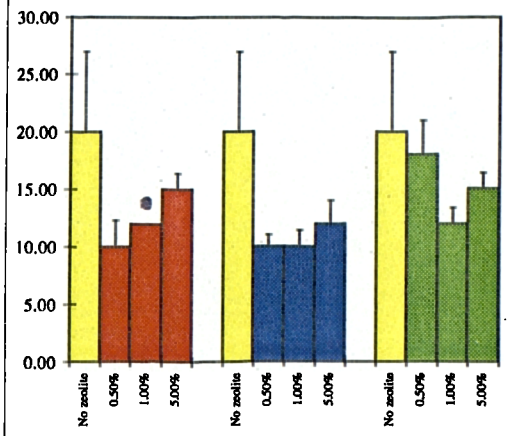
Fig. 7.1. Copper concentration (µg/g dry wt.) for ryegrass foliage grown in Prescot soil amended with zeolites.



Conc.	Zeo P		S.D.	Zeo 4A	S.D.	Zeo Y	S.D.
No zeolite	180		(±2)	180	(±2)	180	(±2)
0.50%	90	c	(±9)	100	(±17)	110	(±17)
1.00%	140	c	(±16)	65	(±10)	90	(±8.5)
5.00%	100		(*)	N.G.	(*)	120	(±3.5)

N.G. = No plant growth; (*) = Single plant survived;(•) S.D.= 0; c=P<0.001.

Fig. 7.2. Copper concentration (µg/g dry wt.) for ryegrass foliage grown in Gateacre soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A	S.D.	Zeo Y	S.D.
No zeolite	20	(±7)	20	(±7)	20	(±7)
0.50%	10	(±2.3)	10	(±1)	18	(±3)
1.00%	12	(±0)	10	(±1.4)	12	(±1.4)
5.00%	15	(±1.4)	12	(±2)	15	(±1.4)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.=0.

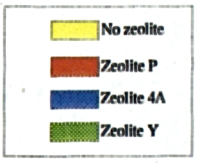
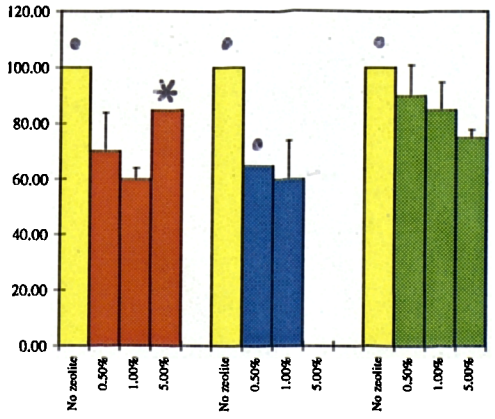


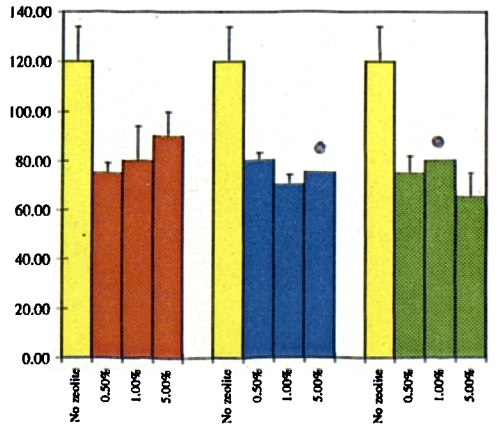
Fig. 7.3. Lead concentration (µg/g dry wt.) for ryegrass foliage grown in Prescot soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A	S.D.	Zeo Y	S.D.
No zeolite	100	(±0)	100	(±0)	100	(±0)
0.50%	70	(±14)	65	(±0)	90	(±11)
1.00%	60	(±4.3)	60	(±14)	85	(±10)
5.00%	85	(•)	N.G.	(•)	75	(±3)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.=0.

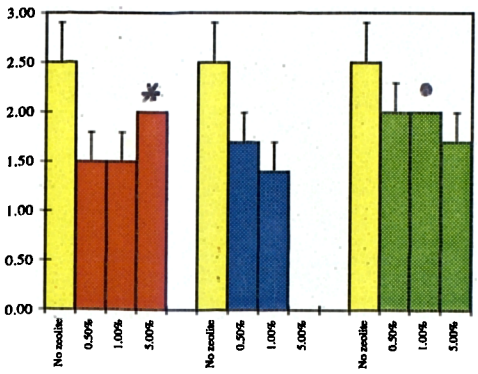
Fig. 7.4. Lead concentration (µg/g dry wt.) for ryegrass foliage grown in Gatacre soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A	S.D.	Zeo Y	S.D.
No zeolite	120	(±14)	120	(±14)	120	(±14)
0.50%	75	(±4.3)	80	(±3)	75	(±7)
1.00%	80	(±14)	70	(±4.2)	80	(±0)
5.00%	90	(±10)	75	(±0)	65	(±10)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.=0.

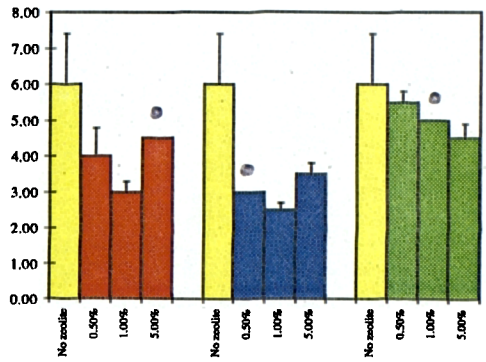
Fig. 7.5.Cadmium concentration (µg/g dry wt.) for ryegrass foliage grown in Prescot soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A	S.D.	Zeo Y	S.D.
No zeolite	2.5	(±0.4)	2.5	(±0.4)	2.5	(±0.4)
0.50%	1.5	(±0.3)	1.7	(±0.3)	2	(±0.3)
1.00%	1.5	(±0.3)	1.4	(±0.3)	2	(±0)
5.00%	2	(*)	N.G.	(*)	1.7	(±0.3)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0.

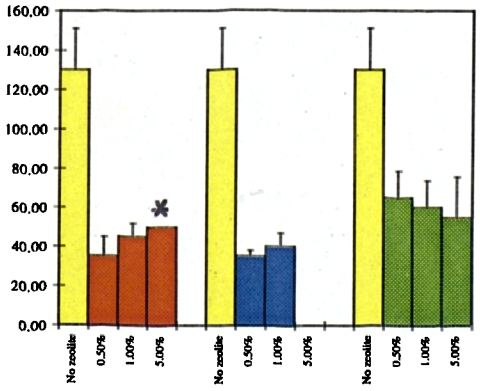
Fig. 7.6.Cadmium concentration (µg/g dry wt.) for ryegrass foliage grown in Gateacre soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A	S.D.	Zeo Y	S.D.
No zeolite	6	(±1.4)	6	(±1.4)	6	(±1.4)
0.50%	4	(±0.8)	3	(±0)	5.5	(±0.3)
1.00%	3	(±0.3)	2.5	(±0.2)	5	(±0)
5.00%	4.5	(±0)	3.5	(±0.3)	4.5	(±0.4)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0.

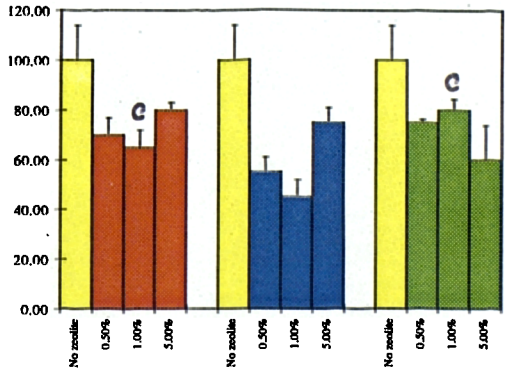
Fig. 7.7. Zinc concentration (µg/g dry wt.) for ryegrass foliage grown in Prescot soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A	S.D.	Zeo Y	S.D.
No zeolite	130	(±21)	130	(±21)	130	(±21)
0.50%	35	(±10)	35	(±3)	65	(±14)
1.00%	45	(±7)	40	(±7)	60	(±14)
5.00%	50	(*)	N.G.	(*)	55	(±21)

N.G. = No plant growth; (*) = Single plant survived; (•) S.D.= 0.

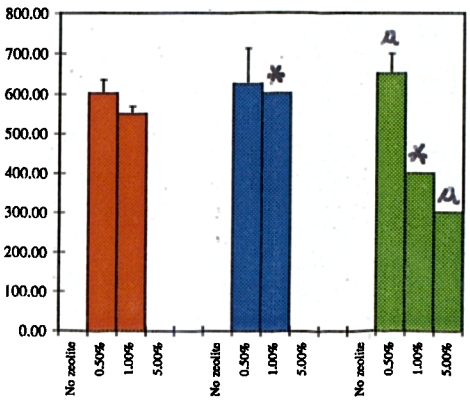
Fig. 7.8. Zinc concentration (µg/g dry wt.) for ryegrass foliage grown in Gateacre soil amended with zeolites.



Conc.	Zeo P		S.D.	Zeo 4A	S.D.	Zeo Y		S.D.
No zeolite	100		(±14)	100	(±14)	100		(±14)
0.50%	70		(±7)	55	(±6)	75		(±1.4)
1.00%	65	c	(±7)	45	(±7)	80	c	(±4.3)
5.00%	80		(±3)	75	(±6)	60		(±14)

N.G. = No plant growth; (*) = Single plant survived; (•) S.D.= 0; c = P < 0.001.

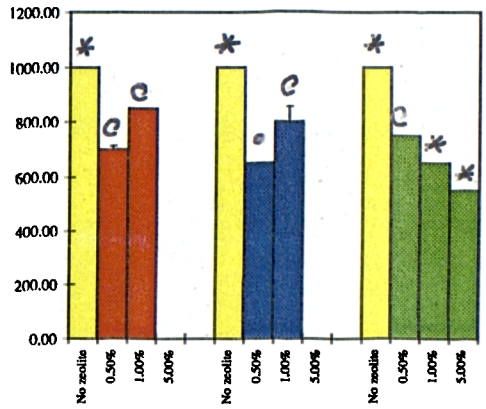
Fig. 7.9. Copper concentration (µg/g dry wt.) for sunflower roots grown in Prescot soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A	S.D.	Zeo Y		S.D.
No zeolite	N.G.	(*)	N.G.	(*)	N.G.		(*)
0.50%	600	(±35)	625	(±88)	650	a	(±50)
1.00%	550	(±18)	600	(*)	400		(*)
5.00%	N.G.	(*)	N.G.	(*)	300	a	(*)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0; a = P < 0.05.

Fig. 7.10. Copper concentration (µg/g dry wt.) for maize roots grown in Prescot soil amended with zeolites.



Conc.	Zeo P		S.D.	Zeo 4A		S.D.	Zeo Y		S.D.
No zeolite	1000		(*)	1000		(*)	1000		(*)
0.50%	700	c	(±14)	650		(±0)	750	c	(±0)
1.00%	850	c	(±0)	800	c	(±57)	650		(*)
5.00%	N.G.		(*)	N.G.		(*)	550		(*)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0; c = P < 0.001.

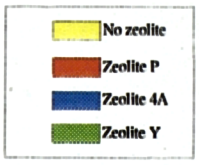
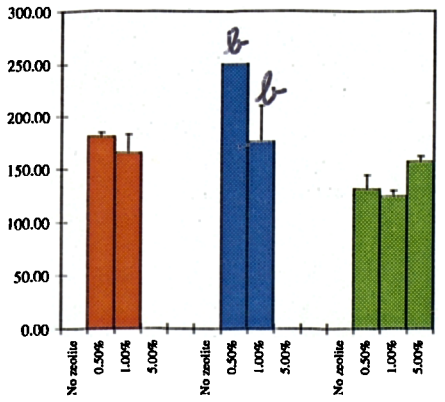


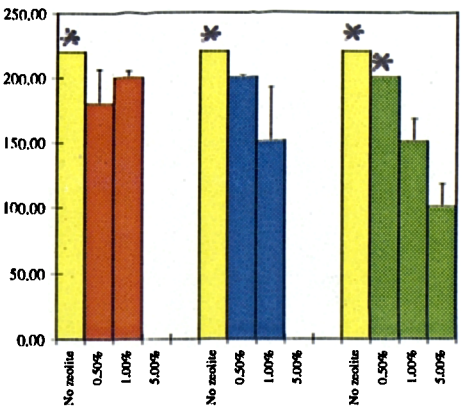
Fig 7.11. Copper concentration (µg/g dry wt.) for sunflower foliage grown in Prescot soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A		S.D.	Zeo Y	S.D.
No zeolite	N.G.	(*)	N.G.		(*)	N.G.	(*)
0.50%	180	(±4.3)	250	b	(±0)	130.5	(±13.4)
1.00%	165	(±17.7)	175	b	(±35.3)	123.8	(±5.3)
5.00%	N.G.	(*)	N.G.		(*)	156.3	(±5.3)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0; b = P < 0.01.

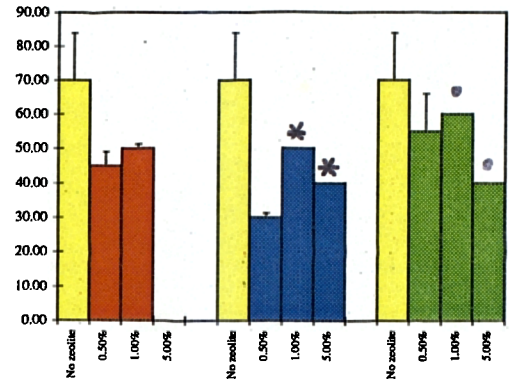
Fig 7.12. Copper concentration (µg/g dry wt.) for maize foliage grown in Prescot soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A	S.D.	Zeo Y	S.D.
No zeolite	220	(*)	220	(*)	220	(*)
0.50%	180	(±26.5)	200	(±1.7)	200	(*)
1.00%	200	(±5.3)	150	(±42.4)	150	(±17.7)
5.00%	N.G.	(*)	N.G.	(*)	100	(±17.7)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0.

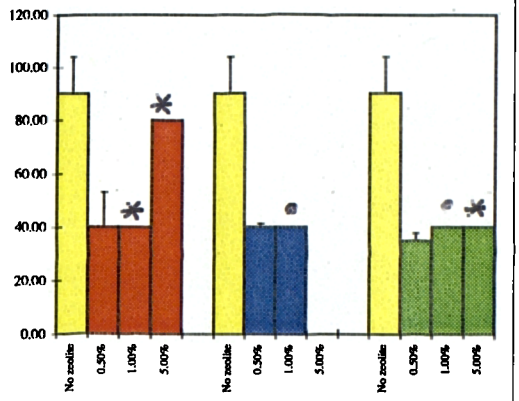
Fig. 7.13.Copper concentration (µg/g dry wt.) for sunflower roots grown in Gateacre soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A	S.D.	Zeo Y	S.D.
No zeolite	70	(±14)	70	(±14)	70	(±14)
0.50%	45	(±4.2)	30	(±1.4)	55	(±11)
1.00%	50	(±1.4)	50	(*)	60	(±0)
5.00%	N.G.	(*)	40	(*)	40	(±0)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0.

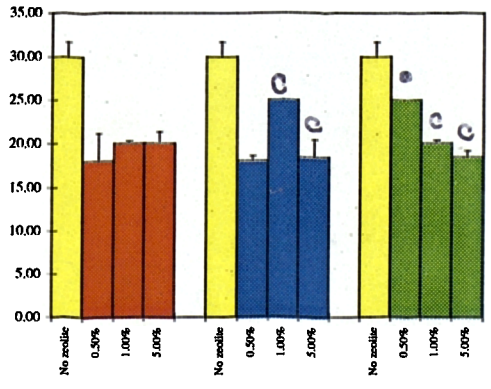
Fig. 7.14. Copper concentration (µg/g dry wt.) for maize roots grown in Gateacre soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A	S.D.	Zeo Y	S.D.
No zeolite	90	(±14)	90	(±14)	90	(±14)
0.50%	40	(±13)	40	(±1.4)	35	(±2.8)
1.00%	40	(*)	40	(±0)	40	(±0)
5.00%	80	(*)	N.G.	(*)	40	(*)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0.

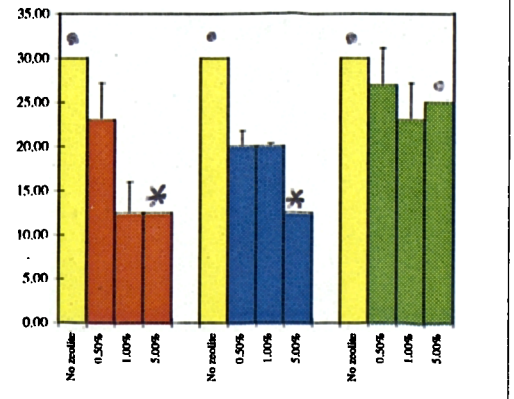
Fig. 7.15.Copper concentration (µg/g dry wt.) for sunflower foliage grown in Gateacre soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A		S.D.	Zeo Y		S.D.
No zeolite	30	(±1.7)	30		(±1.7)	30		(±1.7)
0.50%	18	(±3.2)	18		(±0.6)	25		(±0)
1.00%	20	(±0.3)	25	c	(±0)	20	c	(±0.4)
5.00%	20	(±1.4)	18.5	c	(±2)	18.5	c	(±0.7)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0; c = P < 0.001.

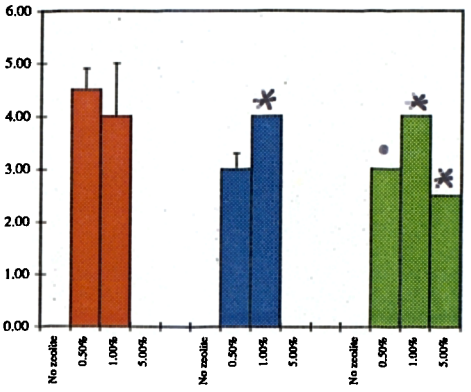
Fig. 7.16. Copper concentration (µg/g dry wt.) for maize foliage grown in Gateacre soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A	S.D.	Zeo Y	S.D.
No zeolite	30	(±0)	30	(±0)	30	(±0)
0.50%	23	(±4.2)	20	(±1.8)	27	(±4.2)
1.00%	12.5	(±3.5)	20	(±0.4)	23	(±4.2)
5.00%	12.5	(*)	12.5	(*)	25	(±0)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0.

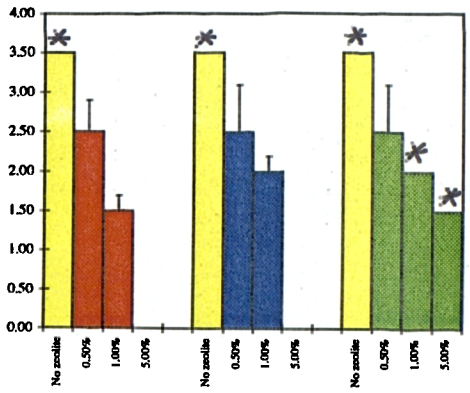
Fig. 7.17.Cadmium concentration (µg/g dry wt.) for sunflower roots grown in Prescot soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A	S.D.	Zeo Y	S.D.
No zeolite	N.G.	(*)	N.G.	(*)	N.G.	(*)
0.50%	4.5	(±0.4)	3	(±0.3)	3	(±0)
1.00%	4	(±1)	4	(*)	4	(*)
5.00%	N.G.	(*)	N.G.	(*)	2.5	(*)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0.

Fig. 7.18. Cadmium concentration (µg/g dry wt.) for maize roots grown in Prescot soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A	S.D.	Zeo Y	S.D.
No zeolite	3.5	(*)	3.5	(*)	3.5	(*)
0.50%	2.5	(±0.4)	2.5	(±0.6)	2.5	(±0.6)
1.00%	1.5	(±0.2)	2	(±0.2)	2	(*)
5.00%	N.G.	(*)	N.G.	(*)	1.5	(*)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0.

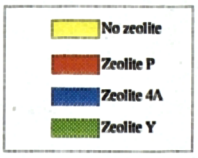
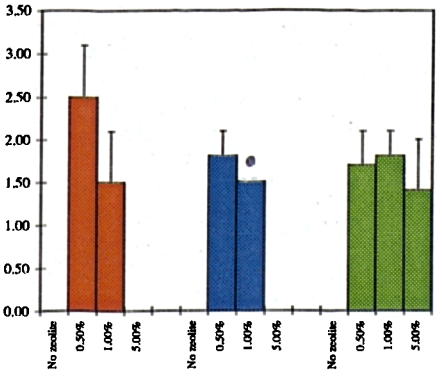


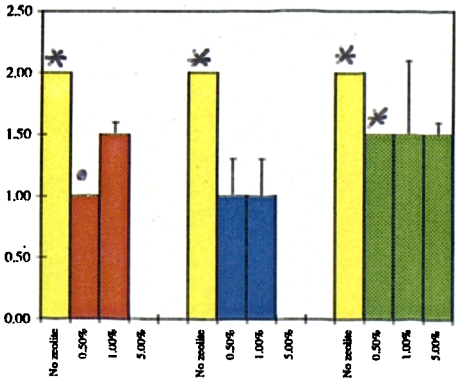
Fig. 7.19. Cadmium concentration (µg/g dry wt.) for sunflower foliage grown in Prescot soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A	S.D.	Zeo Y	S.D.
No zeolite	N.G.	(*)	N.G.	(*)	N.G.	(*)
0.50%	2.5	(±0.6)	1.8	(±0.3)	1.7	(±0.4)
1.00%	1.5	(±0.6)	1.5	(±0)	1.8	(±0.3)
5.00%	N.G.	(*)	N.G.	(*)	1.4	(±0.6)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0.

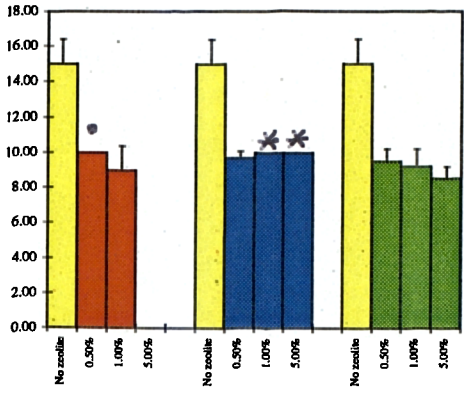
Fig. 7.20. Cadmium concentration (µg/g dry wt.) for maize foliage grown in Prescot soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A	S.D.	Zeo Y	S.D.
No zeolite	2	(*)	2	(*)	2	(*)
0.50%	1	(±0)	1	(±0.3)	1.5	(*)
1.00%	1.5	(±0.1)	1	(±0.3)	1.5	(±0.6)
5.00%	N.G.	(*)	N.G.	(*)	1.5	(±0.1)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0.

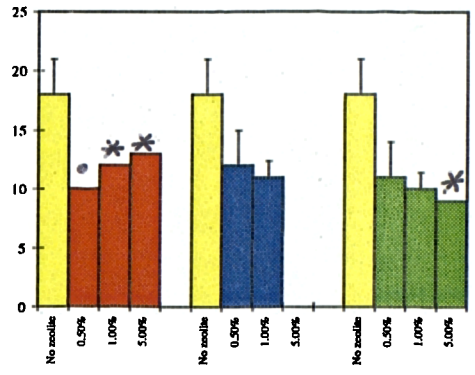
Fig. 7.21.Cadmium concentration (µg/g dry wt.) for sunflower roots grown in Gateacre soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A	S.D.	Zeo Y	S.D.
No zeolite	15	(±1.4)	15	(±1.4)	15	(±1.4)
0.50%	10	(±0)	9.7	(±0.4)	9.5	(±0.7)
1.00%	9	(±1.4)	10	(*)	9.2	(±1)
5.00%	N.G.	(*)	10	(*)	8.5	(±0.7)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0.

Fig. 7.22. Cadmium concentration (µg/g dry wt.) for maize roots grown in Gateacre soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A	S.D.	Zeo Y	S.D.
No zeolite	18	(±3)	18	(±3)	18	(±3)
0.50%	10	(±0)	12	(±3)	11	(±3)
1.00%	12	(*)	11	(±1.4)	10	(±1.4)
5.00%	13	(*)	N.G.	(*)	9	(*)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0.

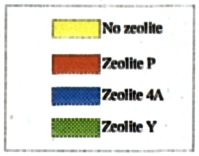
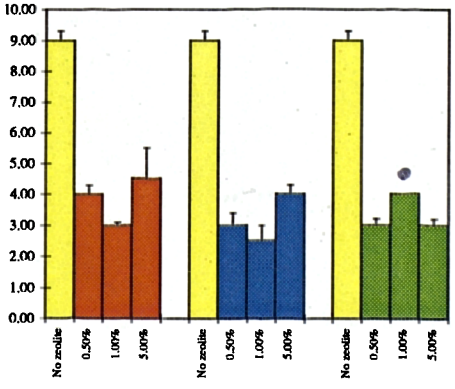


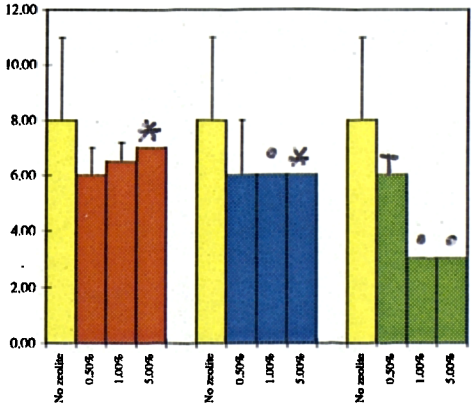
Fig. 7.23.Cadmium concentration (µg/g dry wt.) for sunflower foliage grown in Gateacre soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A	S.D.	Zeo Y	S.D.
No zeolite	9	(±0.3)	9	(±0.3)	9	(±0.3)
0.50%	4	(±0.3)	3	(±0.4)	3	(±0.2)
1.00%	3	(±0.1)	2.5	(±0.5)	4	(±0)
5.00%	4.5	(±1)	4	(±0.3)	3	(±0.2)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0.

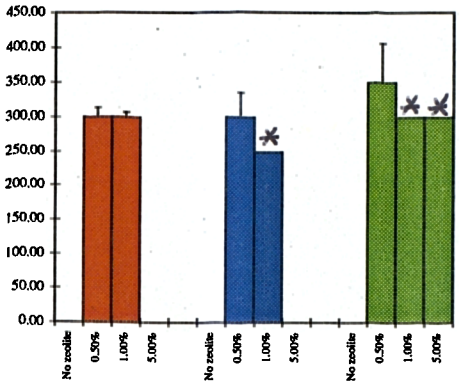
Fig. 7.24. Cadmium concentration (µg/g dry wt.) for maize foliage grown in Gateacre soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A	S.D.	Zeo Y	S.D.
No zeolite	8	(±3)	8	(±3)	8	(±3)
0.50%	6	(±1)	6	(±2)	6	(±1)
1.00%	6.5	(±0.7)	6	(±0)	3	(±0)
5.00%	7	(*)	6	(*)	3	(±0)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0.

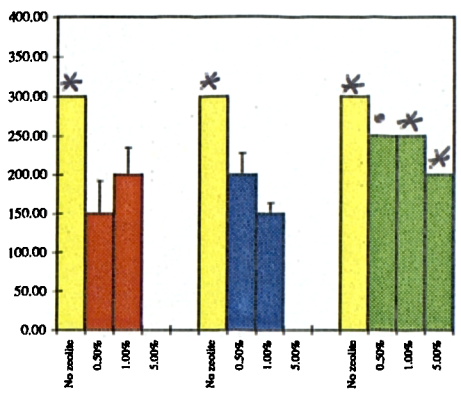
Fig. 7.25. Zinc concentration (µg/g dry wt.) for sunflower roots grown in Prescot soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A	S.D.	Zeo Y	S.D.
No zeolite	N.G.	(*)	N.G.	(*)	N.G.	(*)
0.50%	300	(±14)	300	(±35)	350	(±56)
1.00%	300	(±7.0)	250	(*)	300	(*)
5.00%	N.G.	(*)	N.G.	(*)	300	(*)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0.

Fig. 7.26. Zinc concentration (µg/g dry wt.) for maize roots grown in Prescot soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A	S.D.	Zeo Y	S.D.
No zeolite	300	(*)	300	(*)	300	(*)
0.50%	150	(±42)	200	(±28)	250	(±0)
1.00%	200	(±35)	150	(±14)	250	(*)
5.00%	N.G.	(*)	N.G.	(*)	200	(*)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0.

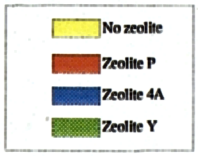
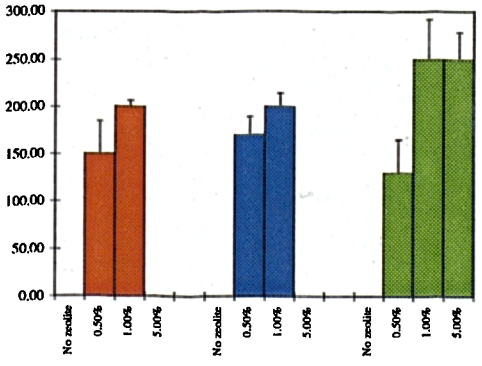


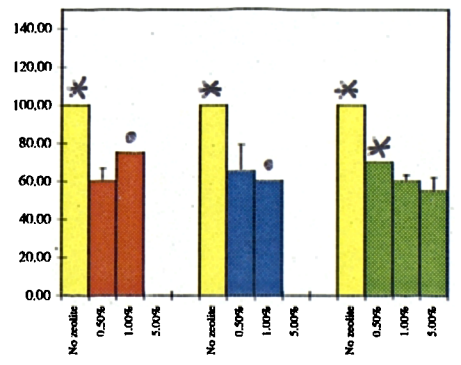
Fig. 7.27. Zinc concentration (µg/g dry wt.) for sunflower foliage grown in Prescot soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A	S.D.	Zeo Y	S.D.
No zeolite	N.G.	(*)	N.G.	(*)	N.G.	(*)
0.50%	150	(±35)	170	(±20)	130	(±35)
1.00%	200	(±7.0)	200	(±14)	250	(±42)
5.00%	N.G.	(*)	N.G.	(*)	250	(±28)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0.

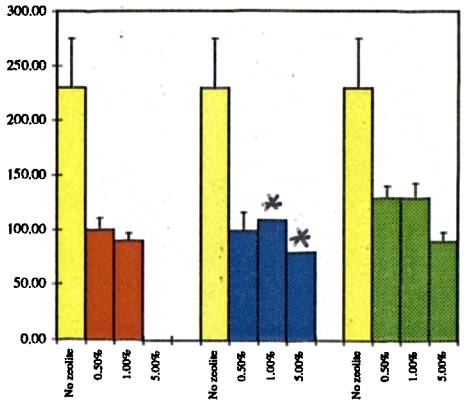
Fig. 7.28. Zinc concentration (µg/g dry wt.) for maize foliage grown in Prescot soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A	S.D.	Zeo Y	S.D.
No zeolite	100	(*)	100	(*)	100	(*)
0.50%	60	(±7)	65	(±14)	70	(*)
1.00%	75	(±0)	60	(±0)	60	(±3.5)
5.00%	N.G.	(*)	N.G.	(*)	55	(±7)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0.

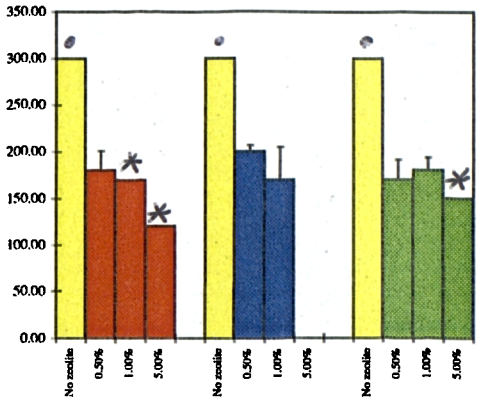
Fig. 7.29. Zinc concentration (µg/g dry wt.) for sunflower roots grown in Gateacre soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A	S.D.	Zeo Y	S.D.
No zeolite	230	(±45)	230	(±45)	230	(±45)
0.50%	100	(±11)	100	(±17)	130	(±11)
1.00%	90	(±7)	110	(*)	130	(±14)
5.00%	N.G.	(*)	80	(*)	90	(±8.5)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0.

Fig. 7.30. Zinc concentration (µg/g dry wt.) for maize roots grown in Gateacre soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A	S.D.	Zeo Y	S.D.
No zeolite	300	(±0)	300	(±0)	300	(±0)
0.50%	180	(±21)	200	(±7)	170	(±21)
1.00%	170	(*)	170	(±35)	180	(±14)
5.00%	120	(*)	N.G.	(*)	150	(*)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0.

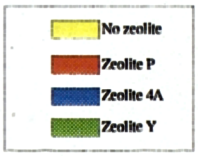
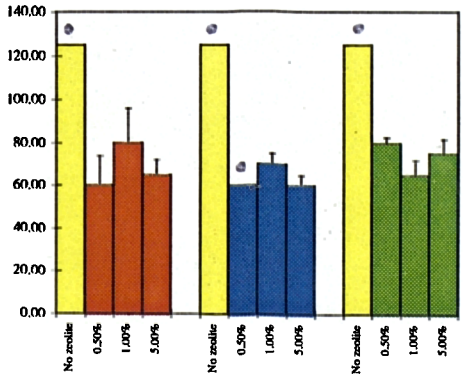


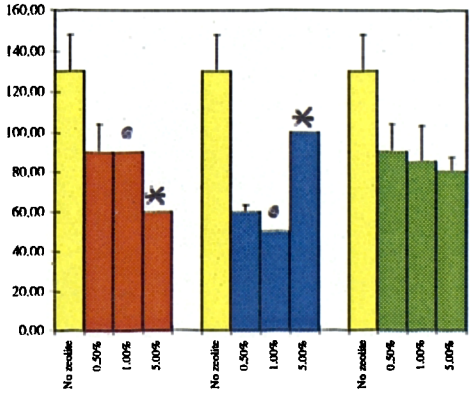
Fig. 7.31. Zinc concentration (µg/g dry wt.) for sunflower foliage grown in Gateacre soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A	S.D.	Zeo Y	S.D.
No zeolite	125	(±0)	125	(±0)	125	(±0)
0.50%	60	(±14)	60	(±0)	80	(±3)
1.00%	80	(±16)	70	(±5)	65	(±7)
5.00%	65	(±7)	60	(±5)	75	(±7)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0.

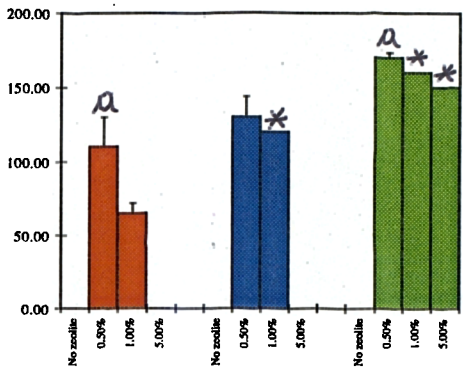
Fig. 7.32. Zinc concentration (µg/g dry wt.) for maize foliage grown in Gateacre soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A	S.D.	Zeo Y	S.D.
No zeolite	130	(±18)	130	(±18)	130	(±18)
0.50%	90	(±14)	60	(±3.5)	90	(±14)
1.00%	90	(±0)	50	(±0)	85	(±18)
5.00%	60	(*)	100	(*)	80	(±7)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0.

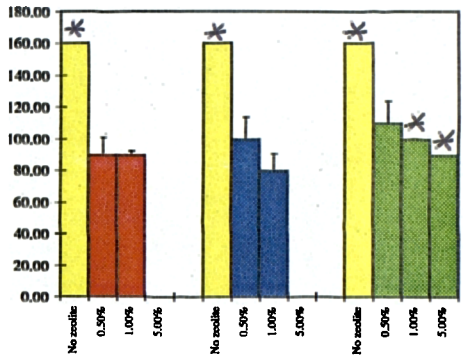
Fig. 7.33. Lead concentration (µg/g dry wt.) for sunflower roots grown in Prescot soil amended with zeolites.



Conc.	Zeo P		S.D.	Zeo 4A	S.D.	Zeo Y		S.D.
No zeolite	N.G.		(*)	N.G.	(*)	N.G.		(*)
0.50%	110	a	(±20)	130	(±14)	170	a	(±3.5)
1.00%	65		(±7)	120	(*)	160		(*)
5.00%	N.G.		(*)	N.G.	(*)	150		(*)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0; a = P < 0.05.

Fig. 7.34. Lead concentration (µg/g dry wt.) for maize roots grown in Prescot soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A	S.D.	Zeo Y	S.D.
No zeolite	160	(*)	160	(*)	160	(*)
0.50%	90	(±11)	100	(±14)	110	(±14)
1.00%	90	(±2.8)	80	(±11)	100	(*)
5.00%	N.G.	(*)	N.G.	(*)	90	(*)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0.

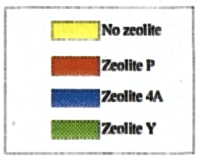
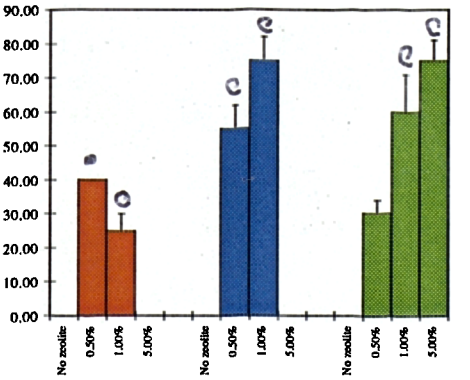


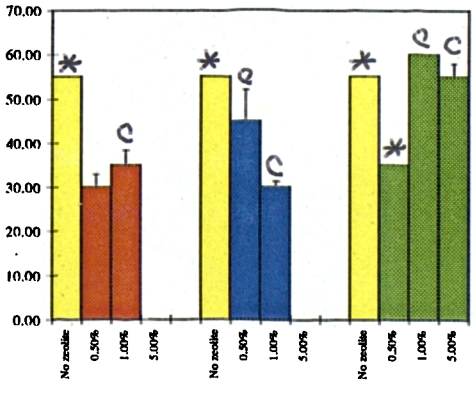
Fig. 7.35. Lead concentration (µg/g dry wt.) for sunflower foliage grown in Prescot soil amended with zeolites.



Conc.	Zeo P		S.D.	Zeo 4A		S.D.	Zeo Y		S.D.
No zeolite	N.G.		(*)	N.G.		(*)	N.G.		(*)
0.50%	40		(±0)	55	c	(±7)	30		(±4)
1.00%	25	c	(±5)	75	c	(±7)	60	c	(±11)
5.00%	N.G.		(*)	N.G.		(*)	75	c	(±6)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0; c = P < 0.001.

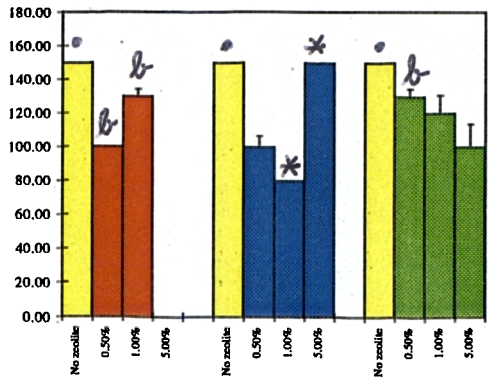
Fig. 7.36. Lead concentration (µg/g dry wt.) for maize foliage grown in Prescot soil amended with zeolites.



Conc.	Zeo P		S.D.	Zeo 4A		S.D.	Zeo Y		S.D.
No zeolite	55		(*)	55		(*)	55		(*)
0.50%	30		(±3)	45	c	(±7)	35		(*)
1.00%	35	c	(±3.4)	30	c	(±1.4)	60	c	(±0)
5.00%	N.G.		(*)	N.G.		(*)	55	c	(±3)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0; c = P < 0.001.

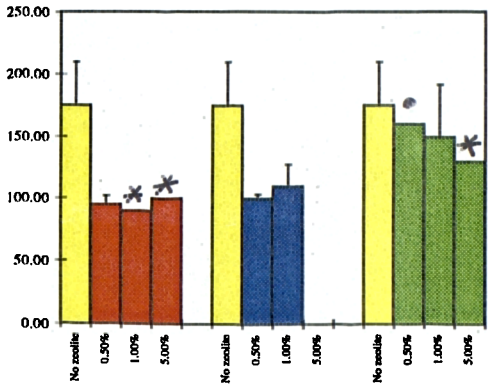
Fig. 7.37. Lead concentration (µg/g dry wt.) for sunflower roots grown in Gateacre soil amended with zeolites.



Conc.	Zeo P		S.D.	Zeo 4A	S.D.	Zeo Y		S.D.
No zeolite	150		(±0)	150	(±0)	150		(±0)
0.50%	100	b	(±0)	100	(±7)	130	b	(±5)
1.00%	130	b	(±4.3)	80	(*)	120		(±11)
5.00%	N.G.		(*)	150	(*)	100		(±14)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0; b = P < 0.01.

Fig. 7.38. Lead concentration (µg/g dry wt.) for maize roots grown in Gateacre soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A	S.D.	Zeo Y	S.D.
No zeolite	175	(±35)	175	(±35)	175	(±35)
0.50%	95	(±7)	100	(±3.5)	160	(±0)
1.00%	90	(*)	110	(±18)	150	(±42)
5.00%	100	(*)	N.G.	(*)	130	(*)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0.

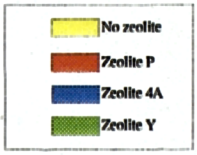
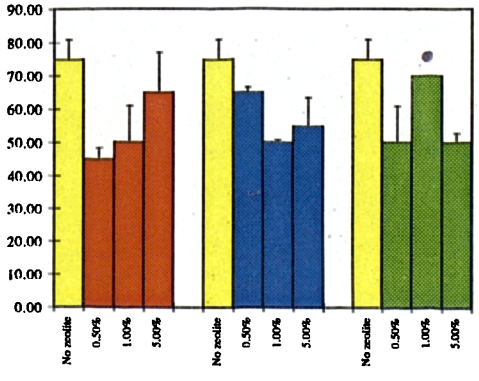


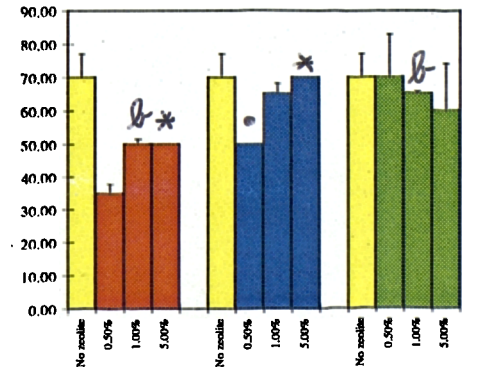
Fig. 7.39. Lead concentration (µg/g dry wt.) for sunflower foliage grown in Gateacre soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A	S.D.	Zeo Y	S.D.
No zeolite	75	(±6)	75	(±6)	75	(±6)
0.50%	45	(±3.4)	65	(±1.4)	50	(±11)
1.00%	50	(±11)	50	(±0.7)	70	(±0)
5.00%	65	(±12)	55	(±8.5)	50	(±3)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0.

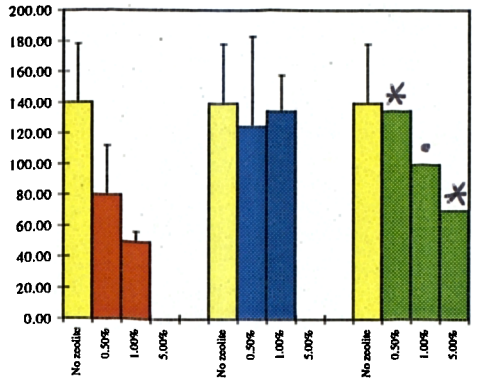
Fig. 7.40. Lead concentration (µg/g dry wt.) for maize foliage grown in Gateacre soil amended with zeolites.



Conc.	Zeo P		S.D.	Zeo 4A	S.D.	Zeo Y		S.D.
No zeolite	70		(±7)	70	(±7)	70		(±7)
0.50%	35		(±3)	50	(±0)	70		(±13)
1.00%	50	b	(±1.4)	65	(±3)	65	b	(±0.7)
5.00%	50		(*)	70	(*)	60		(±14)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0; b = P < 0.01.

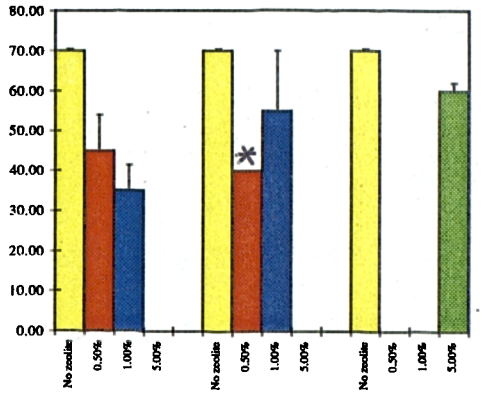
Fig. 7.41. Copper concentration (µg/g dry wt.) for willow shoots grown in Prescot soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A	S.D.	Zeo Y	S.D.
No zeolite	140	(±38)	140	(±38)	140	(±38)
0.50%	80	(±32)	125	(±58)	135	(*)
1.00%	50	(±6.4)	135	(±23)	100	(±0)
5.00%	N.G.	(*)	N.G.	(*)	70	(*)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0.

Fig. 7.42. Copper concentration (µg/g dry wt.) for willow shoots grown in Gateacre soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A	S.D.	Zeo Y	S.D.
No zeolite	70	(±0.5)	70	(±0.5)	70	(±0.5)
0.50%	45	(±9)	40	(*)	N.G.	(*)
1.00%	35	(±6.5)	55	(±15)	N.G.	(*)
5.00%	N.G.	(*)	N.G.	(*)	60	(±2)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0.

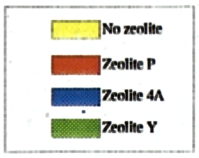
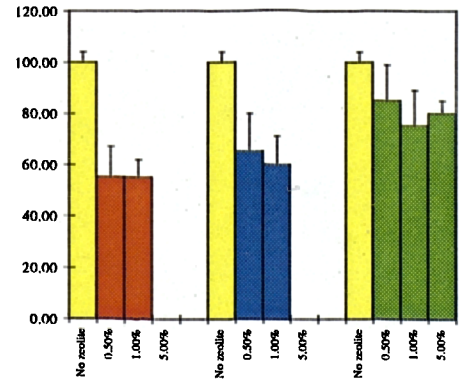


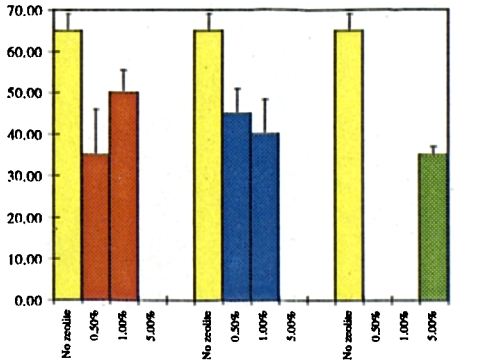
Fig. 7.43. Copper concentration (µg/g dry wt.) for willow leaves grown in Prescot soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A	S.D.	Zeo Y	S.D.
No zeolite	100	(±4)	100	(±4)	100	(±4)
0.50%	55	(±12)	65	(±15)	85	(±14)
1.00%	55	(±7)	60	(±11)	75	(±14)
5.00%	N.G.	(*)	N.G.	(*)	80	(±5)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0.

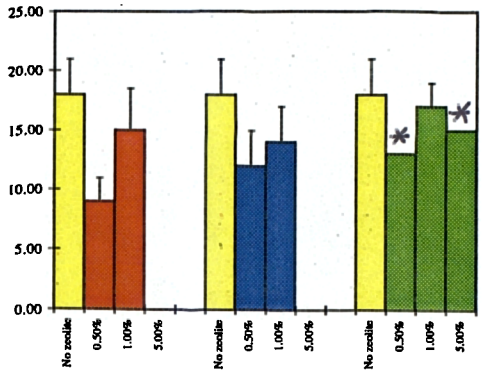
Fig. 7.44. Copper concentration (µg/g dry wt.) for willow leaves grown in Gateacre soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A	S.D.	Zeo Y	S.D.
No zeolite	65	(±4)	65	(±4)	65	(±4)
0.50%	35	(±11)	45	(±6)	N.G.	(*)
1.00%	50	(±5.5)	40	(±8.3)	N.G.	(*)
5.00%	N.G.	(*)	N.G.	(*)	35	(±2)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0.

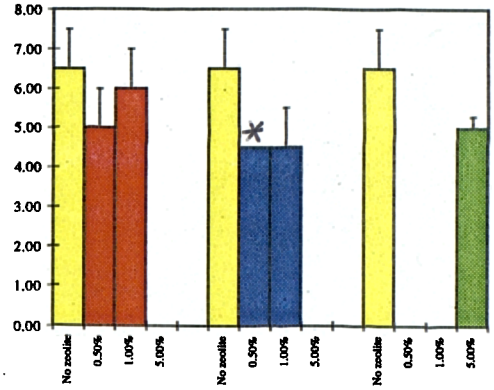
Fig. 7.45. Cadmium concentration (µg/g dry wt.) for willow shoots grown in Prescot soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A	S.D.	Zeo Y	S.D.
No zeolite	18.00	(±3)	18.00	(±3)	18.00	(±3)
0.50%	9.00	(±2)	12.00	(±3)	13.00	(*)
1.00%	15.00	(±3.5)	14.00	(±3)	17.00	(±2)
5.00%	N.G.	(*)	N.G.	(*)	15.00	(*)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0.

Fig. 7.46. Cadmium concentration (µg/g dry wt.) for willow shoots grown in Gateacre soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A	S.D.	Zeo Y	S.D.
No zeolite	6.50	(±1)	6.50	(±1)	6.50	(±1)
0.50%	5.00	(±1)	4.50	(*)	N.G.	(*)
1.00%	6.00	(±1)	4.50	(±1)	N.G.	(*)
5.00%	N.G.	(*)	N.G.	(*)	5.00	(±0.3)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0.

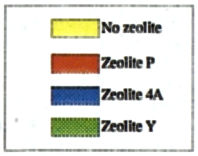
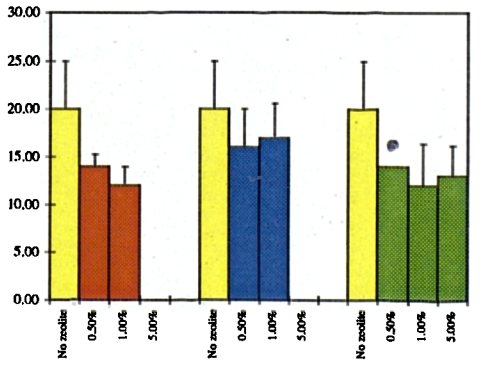


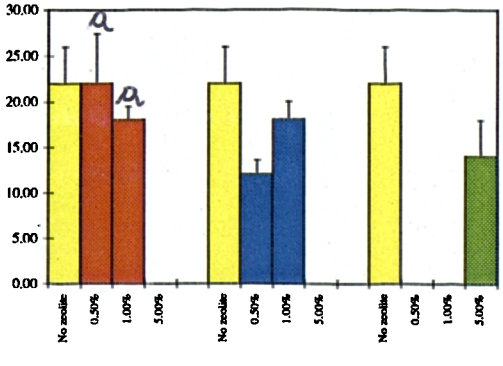
Fig. 7.47. Cadmium concentration (µg/g dry wt.) for willow leaves grown in Prescot soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A	S.D.	Zeo Y	S.D.
No zeolite	20	(±5)	20	(±5)	20	(±5)
0.50%	14	(±1.3)	16	(±4)	14	(±0)
1.00%	12	(±2)	17	(±3.6)	12	(±4.4)
5.00%	N.G.	(*)	N.G.	(*)	13	(±3.2)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0.

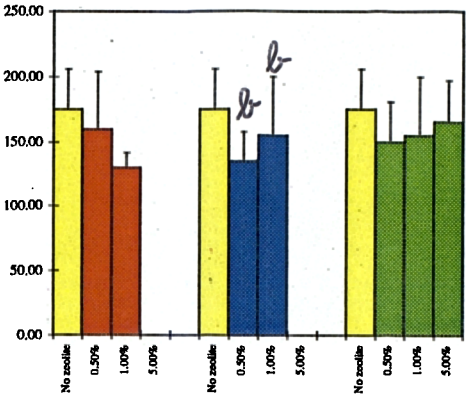
Fig. 7.48. Cadmium concentration (µg/g dry wt.) for willow leaves grown in Gateacre soil amended with zeolites.



Conc.	Zeo P		S.D.	Zeo 4A	S.D.	Zeo Y	S.D.
No zeolite	22		(±4)	22	(±4)	22	(±4)
0.50%	22	a	(±5.4)	12	(±1.6)	N.G.	(*)
1.00%	18	a	(±1.5)	18	(±2)	N.G.	(*)
5.00%	N.G.		(*)	N.G.	(*)	14	(±4)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0; a = $P < 0.05$.

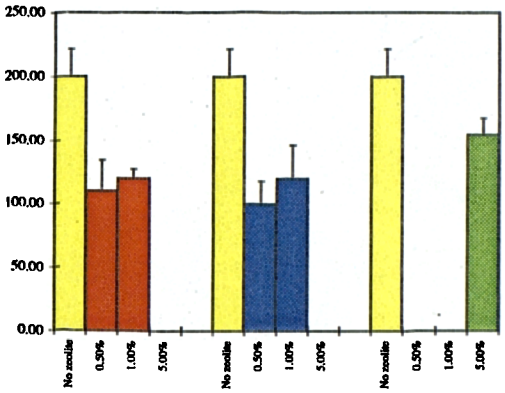
Fig. 7.49. Zinc concentration (µg/g dry wt.) for willow shoots grown in Prescot soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A		S.D.	Zeo Y	S.D.
No zeolite	175	(±31)	175		(±31)	175	(±31)
0.50%	160	(±44)	135	b	(±23)	150	(±31)
1.00%	130	(±12)	155	b	(±45)	155	(±45)
5.00%	N.G.	(*)	N.G.		(*)	165	(±32)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0; b = P < 0.01.

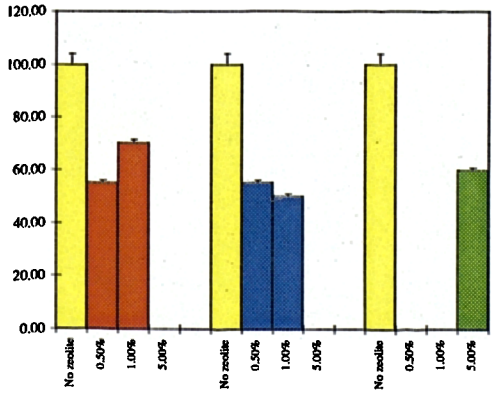
Fig. 7.50. Zinc concentration (µg/g dry wt.) for willow shoots grown in Gateacre soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A	S.D.	Zeo Y	S.D.
No zeolite	200	(±22)	200	(±22)	200	(±22)
0.50%	110	(±25)	100	(±18)	N.G.	(*)
1.00%	120	(±8)	120	(±27)	N.G.	(*)
5.00%	N.G.	(*)	N.G.	(*)	155	(±13)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0.

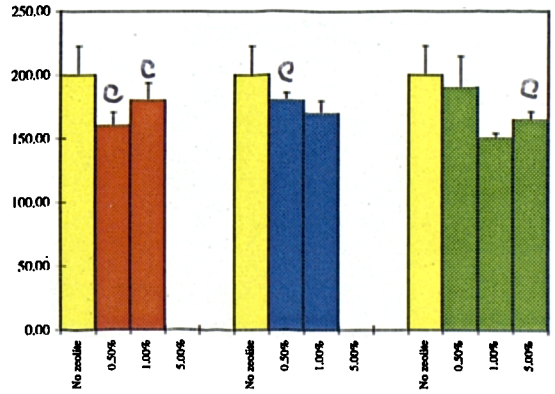
Fig. 7.51. Zinc concentration (µg/g dry wt.) for willow leaves grown in Prescot soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A	S.D.	Zeo Y	S.D.
No zeolite	200	(±23)	200	(±23)	200	(±23)
0.50%	160	(±11)	180	(±6)	190	(±25)
1.00%	180	(±14)	170	(±10)	150	(±4)
5.00%	N.G.	(*)	N.G.	(*)	165	(±6.5)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0.

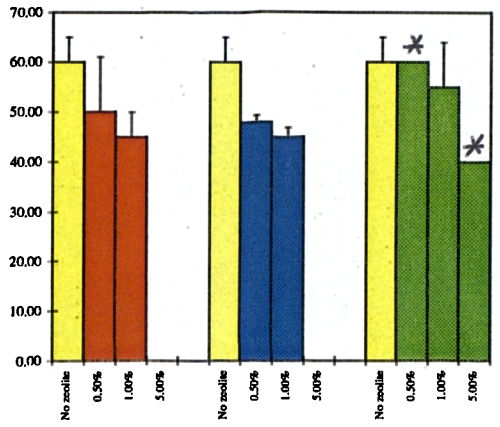
Fig. 7.52. Zinc concentration (µg/g dry wt.) for willow leaves grown in Gateacre soil amended with zeolites.



Conc.	Zeo P		S.D.	Zeo 4A		S.D.	Zeo Y		S.D.
No zeolite	100		(±4)	100		(±4)	100		(±4)
0.50%	55	c	(±1)	55	c	(±1)	N.G.		(*)
1.00%	70	c	(±1.5)	50		(±1)	N.G.		(*)
5.00%	N.G.		(*)	N.G.		(*)	60	c	(±1)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0; c = P < 0.001.

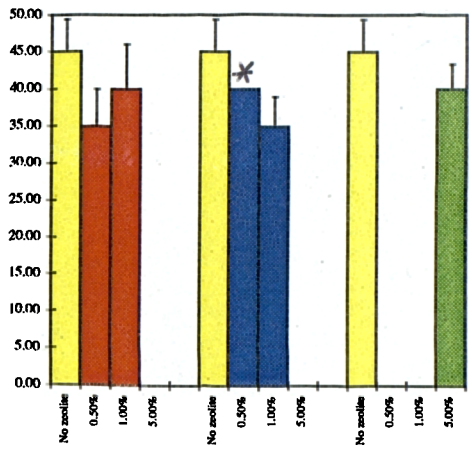
Fig. 7.53. Lead concentration (µg/g dry wt.) for willow shoots grown in Prescot soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A	S.D.	Zeo Y	S.D.
No zeolite	60	(±5)	60	(±5)	60	(±5)
0.50%	50	(±11)	48	(±1.4.)	60	(*)
1.00%	45	(±5)	45	(±2)	55	(±9)
5.00%	N.G.	(*)	N.G.	(*)	40	(*)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0.

Fig. 7.54. Lead concentration (µg/g dry wt.) for willow shoots grown in Gateacre soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A	S.D.	Zeo Y	S.D.
No zeolite	45	(±4.4)	45	(±4.4)	45	(±4.4)
0.50%	35	(±5)	40	(*)	N.G.	(*)
1.00%	40	(±6)	35	(±4)	N.G.	(*)
5.00%	N.G.	(*)	N.G.	(*)	40	(±3.4)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0.

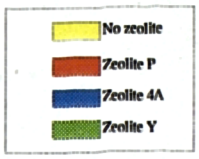
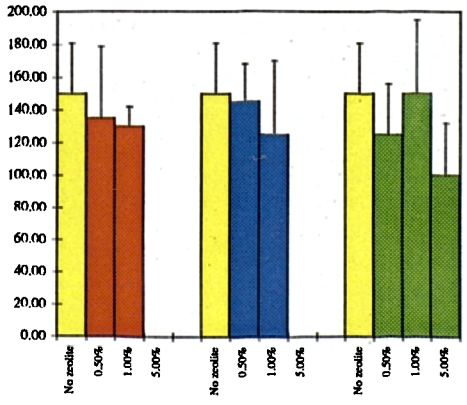


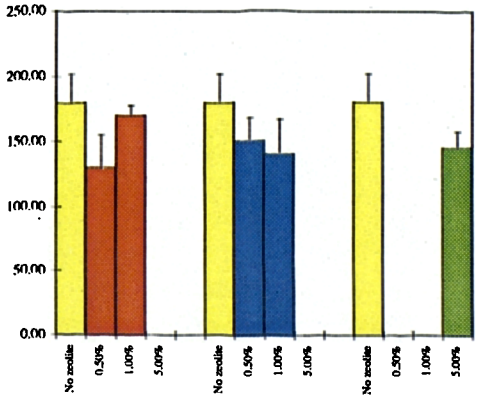
Fig. 7.55. Lead concentration (µg/g dry wt.) for willow leaves grown in Prescot soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A	S.D.	Zeo Y	S.D.
No zeolite	150	(±31)	150	(±31)	150	(±31)
0.50%	135	(±44)	145	(±23)	125	(±31)
1.00%	130	(±12)	125	(±45)	150	(±45)
5.00%	N.G.	(*)	N.G.	(*)	100	(±32)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0.

Fig. 7.56. Lead concentration (µg/g dry wt.) for willow leaves grown in Gateacre soil amended with zeolites.



Conc.	Zeo P	S.D.	Zeo 4A	S.D.	Zeo Y	S.D.
No zeolite	180	(±22)	180	(±22)	180	(±22)
0.50%	130	(±25)	150	(±18)	N.G.	(*)
1.00%	170	(±8)	140	(±27)	N.G.	(*)
5.00%	N.G.	(*)	N.G.	(*)	145	(±13)

N.G. = No plant growth; (*) Single plant survived; (•) S.D.= 0.

Zeolite 4A, when applied at the lowest concentration (0.5%), was very efficient in reducing lead availability; for example, 46% for the shoots of the willows grown on amended Prescot soil (Appendix 4 - Table 4.6.).

Zeolite Y only caused a significant decrease in the bioavailability of the heavy metals to the plants when it was applied at 5% concentration. After adding this zeolite to the contaminated soils, the total uptake of copper decreased by 75% for the foliage of the maize grown on Gateacre soil and for the leaves of the willows grown on Prescot soil. Cadmium uptake decreased even more: 81% for willows and 89% for maize when the application rate of zeolite Y was 5% (Appendix 4 - Tables 4.5. and 4.6.).

Investigations of metal concentrations in the various plant organs indicate that transport of copper and lead to aerial tissues is limited compared to cadmium and zinc. The individual characteristics of the metal elements studied in this work are summarised in Table 4.1. and are of particular significance in relation to accumulation characteristics.

Copper and lead typically forms more stable complexes with organic molecules, and are therefore less mobile once a bond is formed. Cadmium and zinc form less stable complexes and are therefore more mobile; they are taken up into the plant where they can be translocated to the aerial tissues. The greater concentration of cadmium and zinc in aerial tissues may be considered more of a problem because they may move through the food chain more easily. The natural impedances to copper and lead uptake may play a large role in successful induction of resistance and subsequently bioremediation, because these two metals may not enter the symplast.

Copper Behaviour in Test Plants

The decrease in copper availability for plants grown on amended soils was more pronounced for Gateacre than for Prescot soil; for willows, 45 $\mu g\ g^{-1}$ copper in shoots and 35 $\mu g\ g^{-1}$ copper in leaves were found in amended Gateacre soil as compared to 80 $\mu g\ g^{-1}$ copper for shoots and 55 $\mu g\ g^{-1}$ copper for leaves grown on Prescot soil amended with 0.5% zeolite P (Fig. 7.41. and 7.44.). The same pattern was observed and for ryegrass (Fig. 7.1. and 7.2.), and a more significant reduction in the copper available fraction was noticed for maize than for sunflower grown on Gateacre amended soil (Fig. 7.13. and 7.14.).

Uptake of copper was largely confined to the roots, with a lower concentration in the leaf tissue. The highest concentration of root-bound copper was found in maize and sunflower grown in Prescot soil. Statistical analyses of copper concentration showed significant differences for copper uptake between different zeolites (P = 0.000) for maize plants grown in Prescot soil amended with zeolites (Fig. 7.10.), with the order 4A > P > Y at 0.5%.

Copper quickly becomes bound to available sites within the plant and since the first organ encountered is the root system, this typically contains the majority of bound copper. The concentration of copper bound within the roots may prevent further uptake by stunting and reducing the efficiency of the root system. Alloway (1995) notes that uptake of a metal element is not only determined by the quantity of that metal present within the soil solution, but also depends upon the amount of root produced.

Cadmium Behaviour in Test Plants

The most notable reduction in the availability of metals to the plants after adding synthetic zeolites P, 4A or Y to polluted soils was observed for cadmium. The concentration of cadmium taken up from Prescot soil was decreased by zeolites up to nearly 60% in roots and 50% in foliage of maize plants, although there were no significant differences between tissue compartments (Fig. 7.18. and 7.20.). High concentrations of cadmium were measured in the roots of maize grown in unamended Gateacre soil : 18 $\mu g\ g^{-1}$ cadmium as compared to 10 $\mu g\ g^{-1}$ cadmium in 0.5% zeolite P-amended soil (Fig. 7.22.). Sunflower and maize grown in Gateacre soil were found to have a high proportion of cadmium in leaf tissue (9 $\mu g\ g^{-1}$ for sunflower and 8 $\mu g\ g^{-1}$ for maize in unamended Gateacre soil) (Fig. 7.23. and 7.24.). In the presence of synthetic zeolites, the cadmium concentration in sunflower foliage decreased by 72% when Gateacre soil was amended with 1% zeolite 4A and by 67% when 1% zeolite P or 5% zeolite Y was used. Ryegrass grown in Gateacre soil had high cadmium concentrations in the foliage (Fig. 7.6.), ranging from 5.5 $\mu g\ g^{-1}$ cadmium to 2.5 $\mu g\ g^{-1}$ cadmium in zeolite-amended soil. These concentrations are above the normal range of cadmium in plants (0.1 - 2.4 $\mu g\ g^{-1}$).

Zinc Behaviour in Test Plants

There was a more equitable distribution of zinc between tissues compared to the other metals. Maize accumulated the greatest overall concentration of zinc into tissue compartments, with 300 $\mu g\ g^{-1}$ in the roots and 100 $\mu g\ g^{-1}$ in the foliage for unamended Gateacre and Prescot soils (Fig. 7.25.- 7.32.). By adding 0.5% zeolites P or 4A to Prescot soil, the zinc concentration for ryegrass decreased by 73% (Fig. 7.7.). For both roots and foliage, the zinc content of the maize plants grown in amended Prescot soil was considerably decreased after adding synthetic zeolites to this soil. A 50% reduction in the zinc availability was recorded for roots and 40% for foliage when 0.5% zeolites P or 4A were used as amendments, whereas zeolite Y applied at 5% decreased zinc availability by 33% for the roots and 45% for the foliage for Prescot soil (Fig. 7.25. and 7.28.). For Gateacre soil, the reduction in zinc availability after the application of zeolites to this soil was more pronounced for sunflower, with a decrease of 65% for roots and 52% for foliage (Fig. 7.29. and 7.32.). The uptake of zinc differed significantly ($P < 0.001$) for the leaves of the willows grown in Gateacre soil after its amendment with zeolites (Fig. 7.52.).

Lead Behaviour in Test Plants

Elevated concentrations of lead were measured in the roots of all plants grown in the unamended soils (Fig. 7.3., 7.4., 7.33., 7.34., 7.37. and 7.38.). Lead accumulation was greatest in the roots, because lead will precipitate forming either lead phosphate ($Pb_3(PO_4)_2$) or lead carbonate ($PbCO_3$)(Cotter-Howells *et al.*, 1995). The differences between root and foliage lead concentrations were large and greatest in the sunflower. A 6-fold difference between root and foliage of the sunflower grown in Prescot soil was observed. The lead concentration in Prescot soil was 500 $\mu g\ g^{-1}$; lead concentration in the root was 170 $\mu g\ g^{-1}$, while in the foliage 30 μg^{-1}) (Fig. 7.33. and 7.36.). The smallest difference was found in maize (lead at 575 $\mu g\ g^{-1}$ in Gateacre soil resulted in a lead content in the roots of 160 $\mu g\ g^{-1}$ and in the foliage 70 $\mu g\ g^{-1}$) (Fig. 7.37. and 7.40.). Lead uptake was significantly different ($P = 0.000$) between different zeolites, concentrations and plants for amended Prescot soil (Fig. 7.35. and 7.36.).

Lead concentrations decreased in the leaves of the willows grown in Gateacre soil by 28% and 17% respectively, when 0.5% zeolites P and 4A were applied. The willows survived in zeolite Y-amended Gateacre soil only at 5% application rate, and the reduction in the lead content was only 11% for shoots and 19.5% for leaves. Extended field trials are necessary to assess the long term viability of those willows which performed well in this work. The willows could take up significant concentrations of the metals into their tissues to keep soil concentrations low.

Because of their nature, roots and stems can immobilise metallic elements for several years, as opposed to leaves which are shed annually. Accumulation of metals in stems and roots of willows could be of importance because it allows an immobilisation of these elements for long periods of time, therefore reducing risks of contamination by inhibiting the circulation of these pollutants in the environment (Labrecque *et al.*, 1995).

All plants responded to the zeolite treatment, although to different degrees. The observations that the three zeolites studied have greater or lesser effects on the well being of the plants is related to their cation exchange capacities, molecular sieving properties and their efficiency at binding heavy metals in preference to alkali and alkaline earth cations present in the soil.

From observations of the plant growth results (Plates 7.1.c., 7.2.c., 7.3.b., 7.3.c., 7.4.a. and 7.4.b.) and comparable results for the fractionation data (Chapter 5), zeolites 4A and P at 0.50% and 1.00% treatment level were the most effective amendments in terms of increased growth, reduced tissue metal concentrations, and decreased metal uptake. At the same addition level, zeolite P in Prescot and Gateacre soils was more efficient than zeolite 4A. In order to achieve a comparable reduction in metal content of the plant, zeolite Y had to be added at 5%, due to its lower cation exchange capacity per gram of zeolite.

The results suggest a strong reduction of heavy metal availability and consequently phytotoxicity which was confirmed by a marked decrease of the heavy metal content in both roots and foliage of plants grown on zeolite-amended polluted soils. In view of the variable and secondary effects of the heavy metals in the food chain, it is desirable to reduce their concentrations in crops at levels far below the phytotoxic threshold values, since phytotoxicity symptoms sometimes appear at heavy metal concentrations many times higher than the guidelines for animal or human consumption.

7.6.3. Statistical Analysis

Metal concentrations (ppm) were log transformed to achieve approximate normality (Normal plots of residuals were used to check this). There was a significant difference between zeolite type/concentrations ($F_{[9,876]} = 4.36$, $P < 0.001$) and between metal contaminated site ($F_{[3,876]} = 492.83$, $P < 0.001$). There was no significant interaction between zeolite type/concentrations and metal contaminated site ($F_{[9,27]} = 1.06$, $P > 0.05$) (Table 7.5.), so differences between pairs of means for different treatment combinations (e.g., zeolite P at 0.5%, for ryegrass grown in Prescot soil) were tested using Tukey's multiple range test (see Appendix 5). In Fig. 7.1.-7.56., different letters indicate a significant difference at $P < 0.05$; c denotes $P < 0.001$; b denotes $P < 0.01$; a denotes $P < 0.05$, whereas no letter in the tables means there were no significant differences ($P > 0.05$).

Ryegrass grown in Prescot soil accumulated far more copper in the foliage as that grown in Gateacre soil, due to the very high copper level in Prescot soil (Fig. 7.1. and 7.2.). Tukey's test revealed that the addition of zeolite P to Prescot soil, at concentrations of 0.5% and 1% resulted in a major decrease in copper content of the ryegrass in comparison to the unamended soil (see Appendix 5). Although Tukey's test did not indicate any apparent significant differences between means for sunflower (roots and foliage) grown in Gateacre soil amended with synthetic zeolites, the general trend appeared to be a major decrease in cadmium concentration for foliage (Fig. 7.21. and 7.23.) and in zinc concentration for the roots (Fig. 7.29.) after addition of each synthetic zeolite to Gateacre soil.

Table 7.5. Analysis of variance by two-way ANOVA on $\log_{10}$-transformed data.

Source of variation	DF	Seq SS	Adj SS	Adj MS	F	P
Zeolite / Concentration (%)	9	4.97	4.65	0.52	4.36	0.000
Metal contaminated site	3	253.67	175.22	58.4	492.83	0.000
Zeolite / Concentration (%) versus Metal contaminated site	27	3.39	3.39	0.13	1.06	0.383
Error	876	103.8	103.8	0.12		
Total	915	365.8				

Tukey test provides confidence intervals for all pairwise differences between level means. The multiple comparison procedure exemplified by the Tukey test considers the null hypothesis: H_o: $\mu_B = \mu_A$ versus H_A: $\mu_B \neq \mu_A$, where the subscripts denote any possible pair of groups and μ is the population mean.

It is worth pointing out that Tukey's test, like all *a posteriori* methods, is very conservative and will therefore frequently fail to detect a true difference between pairs of means.

For the roots of sunflower and maize plants grown in Prescot soil, zeolite Y at all concentrations appeared to decrease copper concentration significantly (Fig. 7.9. and 7.10.). Zeolite Y showed the same trend for lead concentration in the foliage of maize grown in Prescot soil (Fig. 7.36.). 1% zeolite P and Y had a higher efficiency in reducing zinc concentration in ryegrass grown in Gateacre soil (Fig. 7.8.), whereas for copper in sunflower foliage, zeolites 4A and Y at 1% and 5% gave statistically better results (Fig. 7.15.). All three zeolites showed apparent significant differences between means for lead concentration in sunflower and maize (foliage) grown in Prescot soil amended with zeolites (Fig. 7.35. and 7.36.). For both plants, zeolite P was more efficient when applied at 1% rate, zeolite 4A gave the best results at 0.5% and 1% concentrations, whereas all three concentrations for zeolite Y were equally efficient, as indicated by $P < 0.001$ (Appendix 5).

In agreement with the plant growth and the metal accumulation results for the test plants, Tukey's test indicated that zeolites P and 4A at 0.5% concentration and zeolite Y at 5% decreased zinc availability to the same extent in the leaves of the willow grown in Gateacre soil (Fig. 7.52.). Overall, metal accumulation trends for the test plants showed that plants grown on zeolite-amended soils contained lower metal concentrations than plants grown on unamended soils.

Although there was no general difference between the zeolite concentrations (ANOVA), Tukey tests reveal significant differences for some of the data (such as for copper, cadmium and zinc content of the sunflower foliage grown on amended Gateacre soil, for zinc concentrations in willow, and for lead concentrations in the foliage of maize also grown on Gateacre soil), but not for others (for the foliage of sunflower and maize grown on amended Prescot soil, the probability values for the cadmium content were above 0.4 (see Appendix 5).

Highly significant differences ($P < 0.001$)(also indicated by the letter c in the Tables of Fig. 7.1. - 7.56.) were found with respect to the heavy metal concentrations of test plants as a result of the zeolite addition, for soil types and for all the plants. Concentrations in the test plants were markedly decreased.

Analysis of variance combined with Tukey's test showed that metal concentrations in all the plant species were significantly affected by the application of synthetic zeolites. The addition of zeolites at any rates significantly decrease metal content relative to the unamended soils with significant differences between the three rates. With zeolite P and 4A additions, a significant metal reduction occurred in plant tissues with the increase from 0.5% to 1%, but no further reduction occurred with 5%. For zeolite Y, metal concentration was lower at the highest application rate.

In general, there appeared to be significant differences between soil type, plant tissue and zeolites, with significant interactions between all factors. The statistical analysis results have shown that metal-contamination was fairly a homogeneous distribution.

The analyses clearly show that the introduction of synthetic zeolites into polluted soils leads to a significant reduction in the heavy metal content in plants cultivated on zeolite-amended soils, irrespective of the concentrations of the heavy metals and the zeolite application rate.

Copper in Soils

Ryegrass: For the ryegrass grown in Prescot soil, there were significant differences (from Tukey multiple comparisons test)($P < 0.05$) between zeolite P at 0.5% and 5%, zeolite 4A at 0.5% and 1% and zeolite Y at all concentrations versus the untreated control, and also between 0.5% and 1% zeolite P. For Gateacre soil, a significant contrast was observed only between untreated control and zeolite 4A at 1% concentration ($P = 0.04$).

Sunflower: For sunflower roots in Prescot soil, the contrast was only significant between two concentrations for zeolite Y, namely 0.5% and 5% ($P = 0.044$) whereas the foliage, zeolite 4A at 0.5% concentration gave significant contrasts versus 1% and also versus zeolite Y at all application rates ($P < 0.001$). For Gateacre, the 0.5% zeolite 4A versus untreated control contrast was significant for roots ($P = 0.021$), whereas for the

foliage, all three zeolites at all three concentrations gave significant different contrasts ($P < 0.00$) by Tukey's test and the same contrasts were also noticed between 1% and 5% zeolite 4A and 0.5% and 5% zeolite Y.

Maize: Tukey's test revealed many significant contrasts for maize roots in Prescot soil: zeolites P and 4A at 0.5% and 1% versus untreated control and zeolite Y in all concentrations versus untreated control and each zeolite gave significant contrasts ($P= 0.001$) for different concentrations or versus another zeolite (zeolite P at 0.5% versus 1%; 1% zeolite P versus 1% zeolite Y, 1% zeolite 4A versus zeolite Y at the same concentration and between 0.5% and 5% zeolite Y). For foliage, significant contrasts were found only for 5% zeolite Y versus untreated control ($P = 0.015$). 0.5% and 1% zeolites P and 4A and all concentrations of zeolite Y versus untreated control were significant different for roots in Gateacre soil ($P = 0.015$). For foliage, 1% zeolite P and 5% zeolites P and 4A gave significant differences in comparison to the untreated control ($P = 0.005$).

Willow: No significant differences were obtained for the shoots of willow in Prescot soil. But a significant contrast was found for untreated control versus 0.5% and 1% zeolites P and 4A ($P = 0.002$) for the leaves. The only zeolite which gave a highly significant contrast versus untreated control was zeolite P at 1% for willow shoots in Gateacre soil ($P = 0.021$). For leaves, the significant contrasts were between 0.5% and 1% zeolites P and 4A and 5% zeolite Y versus untreated control ($P = 0.001$), which supports the metal concentration results recorded by AAS measurements regarding the efficiency of each zeolite in decreasing the bioavailable fraction of metals.

Cadmium in Soils

From Tukey multiple comparisons test, no significant contrasts were found for cadmium concentrations in the test plants grown on zeolite-amended soils. The only significant contrasts were obtained in Gateacre soil for sunflower foliage ($P = 0.000$), ryegrass ($P = 0.002$) and for the leaves of willow ($P = 0.03$) and they were mainly between the untreated soil and all zeolites.

Zinc in Soils

Ryegrass: For ryegrass in both soils, significant contrasts for zinc concentration were between all zeolites in all concentrations versus the untreated control ($P < 0.002$).

Sunflower: Tukey's test did not indicate significant contrasts for sunflower grown in Prescot soil, but for Gateacre soil, significant differences ($P < 0.01$) between all zeolites and concentrations versus untreated control were recorded.

Maize: For maize in Prescot soil, the contrasts were only significant for foliage, between 0.5% and 1% zeolites P and 4A and zeolite Y versus the untreated control ($P = 0.001$). The Tukey's test results for maize (foliage) grown in Prescot soil are further proofs of the higher efficiency of zeolites P and 4A when applied at lower concentrations (0.5% and 1%), in comparison to zeolite Y, which had to be applied at higher concentrations in order to produce a similar decrease in the metal availability. In Gateacre soil, significant contrasts were between all zeolites versus untreated control for roots ($P = 0.005$), and between control and zeolite P (5%) and 4A (0.5% or 1%) for foliage ($P = 0.005$).

Willow: For willow (shoots) in Prescot soil, 5% zeolite 4A gave significant contrasts, either versus untreated control or zeolite P ($P = 0.011$). For the shoots of willow in Gateacre soil ($P = 0.000$), the results of Tukey's test were similar to those obtained for copper in the the leaves of willow and also for zinc in the foliage of maize in Prescot soil. Zeolites P and Y versus untreated control gave significant contrasts in the leaves of willow in Prescot soil ($P = 0.005$), whereas in Gateacre soil, significant contrasts were recorded for zeolite P at all concentrations versus the untreated control, for 0.5% and 1% zeolite 4A and for 5% zeolite Y also versus untreated control ($P = 0.000$). Significant contrasts were also noticed between zeolites P and 4A at the same concentrations.

Lead in Soils

Ryegrass: For ryegrass in Prescot soil, significant contrasts were found for 1% zeolite P or 4A against untreated control ($P = 0.024$) and versus all zeolites in all concentrations and untreated control for Gateacre soil ($P = 0.004$).

Sunflower: For sunflower in Prescot soil, Tukey test revealed significant contrasts between 1% zeolite P versus the other two zeolites at the same concentrations (P = 0.021). For the roots of sunflower in Gateacre soil, 0.5% zeolites P and 4A and 5% zeolite Y gave significant contrasts versus the untreated control (P = 0.004), but also 0.5% zeolite P against the other two zeolites. No significant contrasts were recorded for sunflower foliage in Gateacre soil.

Maize: For maize in Prescot soil, 0.5% and 1% zeolite P and 1% zeolite 4A have given significant contrasts versus the untreated control for roots and foliage ($P < 0.05$), and the same zeolites were significantly different compared to zeolite Y. For maize (foliage) in Gateacre soil, the contrasts were between untreated control and 1% zeolite P and also between 1% zeolite Y versus 1% zeolite P (P = 0.01) (from Tukey multiple comparisons test).

Willow: No significant contrasts for shoots were recorded after Tukey's pairwise comparisons. The only significant contrasts were noticed for the leaves in Prescot soil and they were between zeolite 4A and untreated control and also between 0.5% and 5% zeolite 4A (P = 0.01).

7.7. Discussion

Similar patterns are observed when zeolites are used as soil amendments, reducing extractable metal levels in soils polluted from aerial deposition of copper and cadmium from a copper refinery at Prescot and from a sewage-sludge agricultural soil contaminated with cadmium and lead at Gateacre, in Liverpool.

Differences in metal concentrations of test plants were attributed to differential availability of heavy metals in soil, the different lengths of test periods in soils and different zeolite selectivity for heavy metals.

The results for the immobilisation efficiency of synthetic zeolites shown in Fig. 7.1. - 7.56. are a function of the zeolite application rate for the soils contaminated with heavy metals. The experimental results showed that zeolites P and 4A were capable of immobilising heavy metal contaminants in each of the three soils at the lowest treatment rate.

During our investigations, exposure of four plant species to increasing amounts of synthetic zeolites resulted in definite changes in plant available metal fraction. With P and 4A, such changes occurred at low concentrations of the zeolite, with a concomitant improvement of yield and no development of visible signs of phytotoxicity. The effect also became apparent with zeolite Y, which substantially decreased heavy metal content at higher concentration used, and also had a positive influence on yield.

A marked decrease in metal concentrations was observed for all plant parts following zeolite application; metal concentrations were greater in roots than in foliage.

Plant cadmium, zinc, copper and lead availability was considerably reduced in the zeolite-amended soils compared to the controls, despite the high metal content of these polluted soils. The zeolite application does not change the total concentration of the metals in the soils, but their speciation in the soil. The extent of these changes depends on the metal, the zeolite, and the soil type.

These results indicated that the cation exchange reactions known to occur in zeolites influenced plant yields and metal concentrations. The release of sodium cations from binding sites within the mineral structure in exchange for heavy metal ions from solution have permitted the permanent adsorption and retention of these heavy metal ions.

The effects of synthetic zeolites were seen in the higher dry weight yield of test plants from the highly polluted soils and the decreasing bioavailability of zinc, cadmium, copper and lead. Adding synthetic zeolites to the polluted soils had a positive effect on the yield of all crops and resulted in yields higher than the unamended soils for ryegrass, sunflower, maize and willows. Furthermore, biomass production was comparable to that of control compost and in some cases, even higher.

From these results, it was concluded that the phytotoxicity of metal-contaminated substrata can be strongly reduced by addition of synthetic zeolites. The reduced availability of metals in amended soils to plants is achieved because the metals are strongly bound in the zeolite structure. The high metal immobilising capacity of the three synthetic zeolites P, 4A and Y is based on chemical precipitation and ion-exchange.

The successful growth of test plants in zeolite-amended soils combined with the decrease in the concentration of heavy metals measured in the plant tissues is very encouraging and should provide impetus for further research.

7.8. Conclusions

The results presented here demonstrate the efficiency of the three synthetic zeolites in reducing the metal transfer from soil to plants by decreasing the bioavailable fraction of metals and by increasing plant biomass. Consequently, after adding synthetic zeolites to the contaminated soils, smaller amounts of metals will be available for transfer from the first to the second link of the food chain, reducing the risk of reaching animals or humans and also, results in an increase in plant productivity.

Beside synthetic zeolites, other soil amendments are frequently used for decreasing metal bioavailability by increasing the pH of the soils. Remediation by reducing bioavailability is the most widely practiced form of remediation in many regions of the world and is particularly appropriate for trace metal contaminants, such as cadmium, copper, chromium, lead, nickel and zinc. Liming the soil to pH 7 or higher (with calcium carbonate) renders these metals less mobile and unavailable. This method is regularly used in vineyards where copper toxicity occurs as a result of the accumulation of copper from fungicides used previously. The most practicable method of keeping the bioavailability of heavy metals to a minimum in sewage sludge-amended soils is by keeping the pH at around 6.5 or 7. Other methods, often used together with liming, are: to add relatively large amounts of organic matter with the aim of locking-up metals as stable complexes with organic colloids and/or to apply phosphatic fertiliser to stimulate crop growth and precipitate insoluble metal phosphates (Bewley, 1986).

The objectives of the next two chapters were as follows:

1. To examine heavy metal uptake into test plants in response to the following factors: synthetic zeolites (P, 4A and Y), lime, fertiliser (John Innes Base), soil type, and combinations of these.
2. To evaluate each amendment as a potential remedial treatment of soils contaminated with heavy metals.
3. To monitor heavy metal uptake for a greater understanding of the effectiveness of synthetic zeolites in a soil-plant system.

CHAPTER 8
LIME AS A SOIL AMENDMENT

8.1. Introduction

For many centuries lime in various forms (e.g. calcium carbonate, magnesium carbonate, calcium oxide, calcium hydroxide) has been used to raise soil pH in an attempt to improve soil fertility. Lime is applied to land in quantities larger than any other inorganic material (typically 2-10 t/ha), and applications are fairly frequent (typically every 2-5 years) (Davies *et al.*, 1993).

Soils differ considerably in their pH and most temperate crops grow best when soil pH is approximately 6.5 to 7.0. Modification in pH level is usually achieved by the addition of lime or sulphur. The addition of lime decreases the availability of most micronutrients (Wallace *et al.*, 1976), while sulphur has the opposite effect (Edelbauer, 1980). Liming the soil raises the pH, thereby reducing the bioavailability of many heavy metals through increased soil adsorption.

Lime treatment removes heavy metals as insoluble hydroxides, basic salts, or coprecipitated with calcium carbonate or ferric hydroxide. This process does not completely remove mercury, cadmium or lead, so their removal is aided by addition of sulfide, as most heavy metals are sulfide-seekers: $Cd^{2+} + S^{2-} \longrightarrow CdS(s)$. Lime precipitation does not normally permit recovery of metals, and therefore is sometimes undesirable from the economic viewpoint. As most common plants grow best in soil with a pH near neutrality, if the soil becomes too acidic for optimum plant growth, it may be restored to productivity by liming, ordinarily through the addition of calcium carbonate:

$$Soil\}(H^+)_2 + CaCO_3 \longrightarrow Soil\}Ca^{2+} + CO_2 + H_2O$$

Clay minerals exchange cations because of the presence of negatively charged sites on the mineral, resulting from the substitution of an atom of lower oxidation number for one of higher number. Cation exchange in soil is the mechanism by which potassium, calcium, magnesium and essential trace-level metals are made available to plants. When nutrient metal ions are taken up by plant roots, the hydrogen ion is exchanged for the metal ions. This process, plus the leaching of calcium, magnesium, and other metal ions from the soil by water containing carbonic acid, tends to make the soil acidic:

$$Soil\}Ca^{2+} + 2CO_2 + 2H_2O \longrightarrow Soil\}(H^+)_2 + Ca^{2+}(root) + 2HCO_3^-$$

Although liming provides a more than adequate supply of calcium for plants, calcium uptake by plants and leaching by carbonic acid may produce a calcium deficiency in soil. Acid soils may still contain an appreciable level of calcium which, because of competition by hydrogen ion, is not available to plants. In alkaline soils, the presence of high levels of sodium, magnesium, and potassium sometimes produces calcium deficiency because these ions compete with calcium for availability to plants (Manahan, 1994).

Raising the pH by liming is an efficient method for reducing plant absorption of toxic elements and heavy metals. Because of lime's limited solubility, solutions of excess lime do not reach extremely high pH values. The lowest pH level to which an acid soil has to be limed in order to overcome heavy metal contamination is a function of both the type species and concentration of the metal.

Valdares *et al.* (1983) reported that an increase in pH results in a lower uptake of heavy metals, but the effect of liming on the mobility of heavy metals is complex. In acid soils, addition of lime would raise the concentration of exchangeable calcium ions and may release some of the absorbed heavy metals by an ion exchange process (Lakanen, 1967). Further lime application would cause the pH to rise and the availability of heavy metals would decrease due to precipitation. If the soil was rich in organic matter, some of the heavy metals would be present in complexes which may be either more or less readily available than the non-complexed cations.

In addition to lime, sodium hydroxide is also used to increase soil pH to cause metals to precipitate from solution. The hydroxide process is often not as efficient at reducing the availability of heavy metal contaminants to acceptable levels. This is because different metals have minimum solubilities at specific pH's and over addition of hydroxide can cause resolubilisation (Peters and Ku, 1985).

Calcium carbonate also acts as a strong absorbent for heavy metals and can precipitate them as double salts such as $CaCO_3.ZnCO_3$ and $CaCO_3.PbCO_3$ (Ramos *et al.*, 1994). Additionally, when $CaCO_3$ (calcite) precipitates from the soil solution it will coprecipitate metals such as cadmium in the calcite lattice. Calcium and cadmium carbonates have identical crystal structures; cations with ionic radii less than 1 Å (e.g. zinc, copper, cobalt and nickel) can also substitute in the calcite lattice, leading to a significant decrease in the concentrations of these metals in the alkaline solution (Muller, 1994). Calcite has hexagonal (rhombohedral) symmetry and a structure built up of alternate layers of calcium ions (Ca^{2+}) and carbonate groups (CO_3^{2-}) (Reeder, 1983).

Metal mobility and bioavailability are restricted in calcareous soils, due to a combination of chemisorption (Papadopoulos and Rowell, 1988), precipitation of carbonates (Christensen and Tjell, 1984) and competition with calcium ions for absorption sites on plant roots. These processes help to explain the decreased mobility and plant availability normally found when acid soils are limed (Alloway and Jackson, 1991).

Changing soil pH frequently induces large changes in the absorption of trace elements by plants. For example, the zinc content of sorghum plants progressively decreased with increasing pH of Norfolk sandy loam to which calcium carbonate, sodium carbonate or calcium sulphate was added (Wear, 1956). By contrast, the absorption of copper by plants may show remarkably little change with treatments which change soil pH. In these cases it is likely that the behaviour of copper is dominated by the formation of soluble organic complexes in the soil solution, as suggested by Hodgson *et al.* (1966) to explain the insensivity of plants to copper deficiency on calcareous soils.

The increase in soil pH associated with liming improves growing conditions for populations of nitrifying (oxidising) bacteria (White, 1987), which catalyse the oxidation of NH_4^+ to NO_3^- (Haynes, 1984). With this removal of NH_4^+ from the soil and increase in nitrate content, metal uptake into plants could increase, as a result of more soluble metal-nitrate species. An alternative explanation, also relating to microfaunal population changes with liming, is indicated from the work of Johnson *et al.* (1991) who found that some anaerobic microorganisms, (e.g. *Cladosporium sp.* and *Bacillus sp.*) accumulated metals, reducing the availability of metals to plants. A change to a more oxidising environment would cause a depopulation of these species, and hence, a new release of their accumulated metals into soil.

Although the findings are not all consistent, in most cases it is generally found that manipulation of soil pH is an effective and rapid method of controlling the bioavailability of heavy metals in polluted soils (Jackson and Alloway, 1990). In general, the bioavailability of heavy metals in acid soils are reduced by liming to pH 6.5-7 (Alloway and Jackson, 1991), but it is not a permanent solution. Lime is neutralised in soil by acid rain and other acidifying processes, so that subsequent treatments will be needed.

8.2. Experimental

Prescot soil was chosen for this experiment because it has a very low pH of 3.4 and zeolite 4A was chosen because previous experiments (Section 7.6.) showed that (along with zeolite P) it was the most efficient at increasing plant yield and decreasing plant metal concentrations. John Innes Base No.1 potting compost was used as a control.

Both lime and zeolite 4A were applied at the same concentration (0.5%), therefore processed soil (100 g dry wt.) was mixed with 0.5 g of $CaCO_3$ (reagent grade) or zeolite 4A. The mixtures were incubated at room temperature for 7 days. After this time, samples were withdrawn and the pH of the soil was measured using a calibrated Radiometer PHM 85 precision pH meter fitted with a Radiometer GK 2401 C combination glass electrode. Results indicated that 0.5 g of $CaCO_3$ per 100 g of Prescot soil increased soil pH to 4.6, whereas 0.5 g of zeolite 4A per 100 g of Prescot soil increased soil pH to 5.6.

For the growth trials, plastic pots were filled with 4 kg (dry weight basis) of Prescot soil or John Innes No. 1 potting compost (as the control) containing appropriate amendments (Table 8.1.). To ensure that nutrient availability was not a limiting factor, all the pots received the same amount of basal fertiliser (0.16 g John Innes Base Fertiliser per dm^3 soil). After 7 days equilibration, 4 sunflower seeds were sown in each pot. After germination, pots were thinned to 2 plants. In unamended Prescot soil, the sunflower seeds were sown on two successive occasions, but did not germinate. After three months, the remaining plants were harvested and separated into roots and leaves. The roots were washed with distilled water to remove the adherent soil particles. Plant samples were dried at 60°C for 72 hours, weighed and ground in a mechanical sample grinder before digestion. The resulting biomass (fresh and dry) was weighed and recorded.

The plant material was digested following the same procedure as described in Section 7.3. Total lead, zinc, copper and cadmium concentrations were determined in the samples by atomic absorption spectroscopy. Results of triplicate analyses are presented in Tables 8.1. and 8.2.

Table 8.1. Lime and Zeolite 4A effects on yield, tissue metal concentrations, and metal uptake for sunflower roots grown in amended soils (2 plants/pot) (n = 3).

		Roots	Tissue concentrations (mg/kg dry wt.)				Uptake (mg/pot)			
Treatment	pH	Yield (g/pot)	Cu	Cd	Zn	Pb	Cu	Cd	Zn	Pb
Compost (C)	5.9	1.70	15.6 (±0.14)	0.10 (±0.03)	14.5 (±0.71)	11 (±0.00)	0.027	0.0002	0.025	0.019
C+0.5% Lime	6.2	2.22	10.2 (±0.28)	0.06 (±0.01)	11 (±1.41)	10 (±0.00)	0.023	0.0001	0.024	0.022
C+0.5% Zeo4A	6.2	3.12	8.4 (±0.14)	0.04 (±0.00)	10.3 (±0.43)	8.5 (±0.71)	0.026	0.0001	0.032	0.027
Prescot (P)	3.3	N.G.	N.G.	N.G.	N.G.	N.G.	-	-	-	-
P+0.5% Lime	4.6	0.15	212 (*)	2.3 (*)	120 (*)	62.5 (*)	0.032	0.0003	0.018	0.010
P+0.5% Zeo 4A	5.6	0.21	136 (*)	0.6 (*)	76 (*)	50 (*)	0.028	0.0001	0.016	0.011

Note: N.G. = No plant growth; (*) Single plant survived.

Table 8.2. Lime and Zeolite 4A effects on yield, tissue metal concentrations, and metal uptake for sunflower foliage grown in amended soils (2 plants/pot) (n = 3).

		Foliage	Tissue concentrations (mg/kg dry wt.)				Uptake (mg/pot)			
Treatment	pH	Yield (g/pot)	Cu	Cd	Zn	Pb	Cu	Cd	Zn	Pb
Compost (C)	5.9	8.02	3.2 (±0.28)	0.07 (±0.03)	11 (±1.41)	8 (±0.57)	0.026	0.0006	0.088	0.064
C+0.5% Lime	6.2	8.82	1.6 (±0.28)	0.06 (±0.00)	9.0 (±0.71)	5 (±0.00)	0.014	0.0005	0.079	0.044
C+0.5% Zeo4A	6.2	9.49	1.0 (±0.00)	0.05 (±0.00)	8.5 (±0.71)	4 (±0.00)	0.010	0.0004	0.081	0.038
Prescot (P)	3.3	N.G.	N.G.	N.G.	N.G.	N.G.	-	-	-	-
P+0.5% Lime	4.6	1.07	56 (±1.41)	0.46 (±0.03)	31 (±1.41)	29 (±1.41)	0.060	0.0005	0.033	0.031
P+0.5% Zeo 4A	5.6	1.00	28 (±2.83)	0.24 (±0.06)	23 (±2.83)	25 (±0.00)	0.028	0.0002	0.023	0.025

Note: N.G. = No plant growth; (*) Single plant survived.

8.3. Results and Discussion

Addition of synthetic zeolite to soil increases its pH as a result of proton exchange from the soil with the sodium ions in the zeolite cages (Fletcher and Townsend, 1980; Naumova *et al.*, 1994). The net effect of increasing soil pH and the ion exchange properties of zeolites results in a reduction in the amount of soluble or plant available metal ions. Therefore, the effect of zeolite amendments on the phytotoxicity of Prescot soil was compared with the effect of increasing soil pH alone by liming.

Results for sunflower plants grown in compost and Prescot soils treated with lime or zeolite 4A are shown in Tables 8.1. and 8.2. The results indicate that raising soil pH by liming or by adding synthetic zeolites stimulates biomass activity and decreases the amount of heavy metals available for plant uptake.

The dry weights of each treatment were examined for differences between liming or zeolite 4A addition. The dry matter yield for sunflower grown in amended compost increased by 23.4% with lime and 45.5% with zeolite 4A for roots (Table 8.1.) and only by 9% with lime and 15.5% with zeolite 4A for foliage (Table 8.2.). When the same soil amendments were added to Prescot soil, the yield was 0.15 g/pot with lime and 0.21 g/pot with zeolite 4A for roots, and respectively 1.00 g/pot for sunflower foliage. Dry matter yield for a given treatment was almost always higher in the limed soil and soil with the addition of zeolite than in unlimed soil. The yield response observed in the greenhouse experiments when lime was applied is probably due to improved nutrient availability, especially phosphorus (Tagwira, 1988). The positive relationship between plant yield and soil pH is also due to the reduced metal availability caused by precipitation at higher pH. The positive effects of lime or calcareous rich soils on plant growth can additionally attributed to a decrease in soluble aluminium-species, since aluminium is known to be toxic to plants (Hillard *et al.*, 1993).

The effects on the uptake of cadmium, copper, lead and zinc in pH adjusted soils have been evaluated by comparing the decrease in the bioavailable fraction for zeolite- and lime- treated soils in Chapter 6. The present study expands on these observations by determining the actual metal accumulation of sunflower plants grown in pH adjusted soil.

The sunflower parts (roots and foliage) were examined to determine whether the liming treatments had decreased the heavy metal concentrations compared with those grown on the unlimed control soil. Results indicate that as pH increases with liming, heavy metal availability to plants decreases (Tables 8.1. and 8.2.).

In unamended Prescot soil, the test plants did not survive, due to excessive metal toxicity (see Section 7.6.1.). The addition of lime to the soil reduced heavy metal availability, and allowed the sunflower to survive, albeit with elevated levels of metals accumulated in the roots and foliage.

Similar observations have been reported by Jackson and Alloway (1990), who found a cadmium reduction of 43% for cabbage and 41% for lettuce when grown in limed (pH 7) sludge soils, although other studies found that liming did not reduce the cadmium and zinc concentrations in peeled potato tuber (Jackson, 1990) nor the uptake of cadmium by silage maize on either a silt loam or a fine sandy loam (Pepper *et al.*, 1983). Fiskell and Martin (1985) reported that liming increased the uptake of metals by *Lolium multiflorum* as a result of increased dry matter production.

Addition of zeolite 4A to Prescot soil cause an increase in soil pH from 3.4 to 5.6. The concentration of metals in both the roots and foliage of sunflower were greatly reduced, and accompanied by an increase in plant growth as compared with the same quantities of lime used. Addition of lime to compost soil decreased copper concentration by 34.6%, cadmium by 40%, zinc concentration by 24% and lead by 9% for roots, and up to 50% for copper, 14.3% for cadmium, 18% for zinc and 37.5% for lead for foliage. When zeolite 4A was added to compost soil, the metal concentrations in roots were decreased by 46% for copper, 60% for cadmium, 29% for zinc and 23% for lead, whereas in the foliage, the order of decreasing metal concentration was: copper (69%) > lead (50%) > cadmium (28.6%) > zinc (23%).

Lime and zeolite 4A were used simultaneously as soil amendments so that a direct comparison of the immobilisation efficiency could be made between the two treatments. However, although both treatments increased soil pH, 0.5% zeolite 4A provided superior immobilization efficiencies to lime (Tables 8.1. and 8.2.). The addition of lime at the same concentration produced a similar effect, although the differences were not as great as those for zeolite 4A. The efficiency of zeolite 4A compared to that of lime in reducing heavy metal concentrations in amended Prescot soil was above 36% for copper and zinc, 74% for cadmium and 20% for lead in sunflower roots, and above 48% for copper and cadmium, 26% zinc and 14% lead in foliage.

The addition of lime to soils will result in an increase in the concentration of calcium, making it the dominant cation over the metal ions in the soil, increasing it's competitiveness at the soil/root interface, and increasing the pH will cause precipitation of metals (Hodkinson, 1994). Since the leaching of trace metals from soil is a slow

process, lime application serves as an inhibitor towards uptake of such metals in toxic concentrations by plants. It is not known how long the effects of liming will persist, or how lime application will alter ecosystem processes on a long-term basis.

Although zeolites, like lime, increase soil pH (therefore reducing the availability of heavy metals to the plants), the concentration of heavy metals in sunflower plants grown in zeolite 4A amended Prescot soil was significantly less than for sunflowers in limed Prescot soil. This is attributed to the slightly higher pH and additional ion-exchange property of synthetic zeolites.

Extrapolation from the glasshouse to the field involves many uncertainties. The lime was thoroughly mixed with all the soil in each pot whereas in the field it would be applied to the soil surface. In the field, leaching of calcium would cause progressive soil acidification and this will in turn affect the bioavailability of the heavy metals. Often this acidification would be corrected by more lime, but further lime applications might not be made for several years or even decades if land is taken out of agricultural use. Plant roots also exploit soils in pots more effectively than in the field (Davies *et al.*, 1993). Quantifying the effects of soil acidity on plant growth remains a challenging research topic as numerous soil and plant growth factors are influenced by pH and lime.

Although both soil amendments, synthetic zeolites and lime, increase the pH of the soil, therefore decreasing metal bioavailability, the ion-exchange properties of the zeolites make them more effective soil amendments, as the bioavailable fraction of metals is significantly decreased by ion-exchange processes. These results are in agreement with those presented in Chapter 6. As previously mentioned, lime needs to be applied quite frequently in order to maintain its effect on metal availability, whereas a single application of synthetic zeolites is sufficient to lock up heavy metals permanently.

CHAPTER 9

JOHN INNES BASE FERTILISER AS A SOIL AMENDMENT

9.1. Introduction

Fertiliser additions may also affect the uptake of heavy metals and, therefore, constitute a significant external factor. Phosphate fertilisers have been shown to decrease availability of heavy metals (by precipitating the metals, for example lead as lead phosphate $Pb_3(PO_4)_2$ to plants in some (e.g. Street *et al.*, 1978), but not all pot experiments (e.g. Sanders & Kherbawy, 1987).

Application of macronutrients (like phosphorus) or metallic elements (like potassium or iron) may revitalise plants grown in polluted soils, but they must be used with a particular care because of complex relations between all mineral compounds and soil organic matter for different soil types (Kiekens *et al.*, 1984). Moreover, data obtained for *in vivo* experiments cannot be simply transferred to natural conditions because they differ with regard to plant sensitivity and growth stage (Reboredo, 1994). Even the choice of plant organs for monitoring changes in the physiological processes may be important because of differences in susceptibility to heavy metals and their distribution mechanisms (Sieghardt, 1990).

Additions of fertiliser cations and anions such as potassium, ammonium or nitrate, which are necessary for most pot experiments, may also affect the solubility of heavy metals in soil. The increase in the concentrations of ions in solution is accompanied by a decrease in soil pH. Crop fertilisers contain nitrogen, phosphorus, and potassium as major components. Fertilisers are designated by numbers, such as 6 - 12 - 8, showing the respective concentrations of nitrogen expressed as N (6%), phosphorus as P_2O_5 (12%) and potassium as K_2O (8%). Farm manure corresponds to an approximately 0.5 - 0.24 - 0.5 fertiliser. Organic fertilisers such as manure must undergo biodegradation to release the simple inorganic species (NO_3^-, $H_xPO_4^{x-3}$, K^+) assimilable by plants. Anhydrous ammonia has a very high nitrogen content of 82%. It may be added directly to the soil, for which it has a strong affinity because of its water solubility and formation of ammonium ion:

$$NH_3\ (g)\ (water) \longrightarrow NH_3\ (aq)$$

$$NH_3\ (aq) + H_2O \longrightarrow NH_4^+ + OH^-$$

Special equipment is required, however, because of the toxicity of ammonia gas. Ammonium-nitrate is a common solid nitrogen fertiliser and although convenient to apply to soil, it requires considerable care during manufacture and storage because it is explosive. Potassium fertiliser components consist of potassium salts, generally KCl. One problem encountered with potassium fertilisers is the luxury uptake of potassium by some crops, which absorb more potassium than is really needed for their maximum growth (Manahan, 1994).

Bioavailability of nutrients is dependent on their concentration in the soil solution, on exchange equilibria between solution and adsorbed nutrient species, and uptake by plants of nitrogen, potassium and magnesium is closely linked to the rate of transpiration (Lorenz *et al.*, 1994). Available, cost-effective means of providing soil supplements must be determined and assessed in terms of moisture requirements and the establishment of fertiliser rates, so that adequate vegetative cover and growth responses are possible.

The combination of fertiliser and zeolite as a soil amendment has a number of advantages. In addition to the zeolite properties of metal immobilisation, they can act as reservoirs for the slow release of fertiliser ions such as potassium and ammonium, and also prevent loss of nitrogen by volatilisation from soil surfaces.

9.2. Experimental

This experiment was designed to investigate the effect on grass growth due to the combined applications of zeolite 4A with different levels of fertiliser.

Trelogan soil was chosen for this experiment, because of its low fertility and low organic matter content. John Innes No.1 potting compost was used as a control. Zeolite 4A was applied at 1.00% w/w dry weight, which was shown to be an optimum concentration for improving plant biomass and metal concentrations (Section 7.6.).

Plastic trays (20 cm square) were used. Each tray contained 1 kg of the unamended or amended soil and appropriate amount of fertiliser was added to each tray. The composition of fertiliser is given in Table 9.1.

The amount of fertiliser and zeolite added to the Trelogan soil experiments are given in Table 9.2. The currently recommended fertiliser rate of John Innes Base Fertiliser (0.16 g kg^{-1} soil) was also increased by 10 and 20 times respectively. Each treatment was thoroughly mixed and each tray was filled to within 1 cm of the rim. The

Table 9.1. Composition of John Innes Base Fertiliser

Compound fertiliser	5.1 - 8.2 - 10
Total Nitrogen (N)	5.1%
Total Phosphorous Pentoxide (P_2O_5)	8.2% (3.5% P)
Phosphorous Pentoxide (P_2O_5) soluble in water	7.2% (3.1% P)
Phosphorous Pentoxide (P_2O_5) insoluble in water	1.0% (0.4% P)
Total Potassium Oxide (K_2O)	10.0% (8.3% K)

soils were allowed to equilibrate for 7 days in the glasshouse. Ryegrass (*Lolium perenne*, var. Elka) was sown at 0.5 g per tray 0.5 cm below the soil surface in a uniform arrangement. All the trays were watered thoroughly, arranged randomly and covered with a plastic sheet until germination commenced. The plastic was removed after germination and the plants watered every day for 6 weeks. The temperature was maintained at not less than 15°C and natural light was supplemented to give a day length of approximately 15 hours. The arrangement of trays was re-randomised every three to four days.

The resulting foliage biomass was cut after 6 weeks. The grass was cut 2 cm above the soil surface and dry weight yields were recorded.

Lead, zinc, copper and cadmium concentrations in the foliage were determined by atomic absorption spectroscopy using a single beam, background corrected instrument. Results of duplicate analysis are presented in Table 9.2.

9.3. Results and Discussion

Ryegrass sown in unfertilised Trelogan soil which was either amended or not with zeolite 4A at 1% did not survive. The seeds germinated, but chlorosis and drying started immediately and plants died in less than one week after germination.

The dry matter yields of ryegrass treated with three different combinations of fertiliser and zeolite 4A are presented in Table 9.2.

The addition of fertiliser to Trelogan soil improved the fertility of the soil, as evidenced by the survival of ryegrass. It was also observed that there was not a significant difference in the dry weight of the harvested ryegrass after six weeks which

Table 9.2. Fertiliser and Zeolite 4A effects on yield, tissue metal concentrations, and metal uptake for ryegrass foliage grown in amended soils (2 trays/treatment) (n = 2).

Treatment	Yield (g//trays)	Tissue concentrations (mg/kg dry wt.)				Uptake (mg/treatment)			
		Cu	Cd	Zn	Pb	Cu	Cd	Zn	Pb
Compost (control)	0.685	9 (8.5-9.5)	0.03 (0.03-0.03)	22.5 (20-25)	5 (5-5)	0.062	0	0.015	0.003
Trelogan (control)	N.G.	N.G.	N.G.	N.G.	N.G.	-	-	-	-
Trelogan (1F)	0.250	48 (46-50)	14 (13-15)	370 (365-375)	200 (200-200)	0.012	0.004	0.093	0.050
Trelogan (10F)	0.260	50 (50-50)	20 (20-20)	430 (420-440)	240 (230-250)	0.013	0.005	0.112	0.062
Trelogan (20F)	0.365	60 (60-60)	22 (20-24)	500 (500-500)	350 (340-360)	0.022	0.008	0.182	0.128
Trelogan + 1% Zeo 4A	N.G.	N.G.	N.G.	N.G.	N.G.	-	-	-	-
Trelogan + 1% Zeo 4A (1F)	0.425	27 (25-29)	7.5 (7-8)	220 (215-225)	135 (130-140)	0.012	0.003	0.094	0.057
Trelogan + 1% Zeo 4A (10F)	0.425	32 (30-34)	8 (7.5-8.5)	250 (250-250)	165 (160-170)	0.014	0.004	0.106	0.070
Trelogan + 1% Zeo 4A (20F)	0.500	40 (40-40)	10 (10-10)	300 (300-300)	235 (225-245)	0.020	0.005	0.150	0.117

Note: F = Fertiliser added at normal rate (1F) and at increased rates (10F) and (20F) respectively; N.G. = No plant growth.

had been treated with either the normal fertiliser application rate or 10 and 20 times the normal application rate (Table 9.2.).

The plants treated with zeolite 4A and a single dose of fertiliser had greater yields than those treated with increasing rates of fertiliser alone. The addition of increasing amounts of fertiliser to the zeolite 4A treated soils did not increase the dry weight of ryegrass compared to the single dose application.

It has been shown that the combined addition of fertiliser and zeolite to improve soil fertility and reduce plant metal concentrations is successful. Nevertheless, for field situations where fertilisers can reach locally high concentrations, this may lead to a mobilisation of heavy metals and, therefore, increased bioavailability and toxicity.

Zeolites can be prepared through exchange with nutrient ions such as ammonia and potassium and act as a reservoir, holding such elements in their structure for slow release to the soil solution and hence to the plants. This will increase the overall efficiency of such additives and reduce the total cost of fertilisation.

The heavy metal content and the dry weight of the ryegrass which was harvested after six weeks was determined after digestion with nitric acid and analysed by AAS. Results are presented in Table 9.2.

The results indicate that the concentration of all metals analysed from the ryegrass grown in fertiliser amended soil had very high metal concentrations compared to the compost control. The concentrations of the metals accumulated by the ryegrass grown in fertiliser amended zeolite soils are far in excess of acceptable limits for metals in plants (see Table 4.5.).

Ryegrass grown in Trelogan soil which had been amended with both zeolite 4A and fertiliser added at the normal rate showed a large reduction in metal concentrations as compared with ryegrass grown in soil which had been amended with only fertiliser. A combination of those two amendments decreased copper concentration up to 44%, cadmium concentration by 46%, zinc concentration over 40% and lead concentration up to 33%.

Increasing the amount of fertiliser added to the zeolite amended soil resulted in an increase of metal content of the ryegrass from 27 to 40 ppm for copper, from 7.5 to 10 ppm for cadmium, from 220 to 300 ppm for zinc and from 135 to 235 ppm for lead. This increase indicates an increased availability of heavy metals in Trelogan soil and can be attributed to two principal factors, namely (1) an increased competition for the ion

exchange sites in the zeolites resulting from increased potassium and sodium content, and (2) solubilisation of the heavy metal by the nitrogen component of the fertiliser.

After applying lime or compost to polluted soils, the bioavailable fraction of metals decreased due to an increase in soil pH. The combined zeolite effects of pH increase and ion-exchange processes resulted in a considerably decrease in heavy metal availability. All the other soil amendments (John Innes Base Fertilizer, lime or compost) provided supplementary, but rather limited metal-binding capacity compared to the immobilising capacity of the synthetic zeolites. The four types of immobilising agents proved to be effective in different degrees in mitigating the metal uptake by plants. Among the tested additives, synthetic zeolites exhibited the most promising potential in reducing metal transfer from the soil to the soil solution and their eventual entry into the food chain via plant uptake.

CHAPTER 10.
FIELD TRIAL AT PRESCOT.

10.1. Introduction

Vegetative growth on mine waste and metal contaminated land is characteristically patchy and varied; isolated areas can become destabilised and removed by wind and water action. This problem can be overcome by creating a continuous self-sustaining cover (Mitchell and Atkinson, 1991). This is considered to be the most effective and, in the long term, the cheapest method of controlling wind and water borne pollution from metalliferous contamination. In addition this approach improves the visual impact of the sites, particularly when vegetated with a wide range of common local plant species. Before successful revegetation can occur however, the hostile chemical, physical and biological characteristics of the spoil must be overcome. The proposed method of land reclamation involves the control of conditions to allow self-sustaining vegetation to become established. Several methods of ameliorating the conditions found in contaminated site were investigated (Chapters 7, 8, 9). The most promising of these was the *in situ* use of ion-exchanging materials. An *in situ* approach has the greatest flexibility and can be used on sites where large mechanical plant necessary to reprocess the waste cannot gain access, or in areas that have undergone partial natural regeneration.

In applying the amendments to contaminated land it is important to emphasise that no specialised equipment is required, the amendment is ploughed into the land to a depth equivalent to the rooting layer. Disturbance of the land is minimised so as not to expose any remaining sulphide minerals at depth to oxidation. To speed up revegetation, seeding, possibly with a nutrient supply being made available, may be needed. Once the vegetation cover is established, the objective of the novel restoration technique is to reduce, permanently, the availability of toxic metals to the root systems (Atkinson, 1994).

Laboratory and greenhouse experiments described throughout this work have demonstrated that the use of synthetic zeolites as amendments for polluted soils results in a significant decrease of the bioavailability of the heavy metals. There are many difficulties in extrapolating laboratory experiments to field conditions, particularly if the great differences of growing and climatic conditions or application techniques are considered.

In general, the uptake of metals from soils is greater from plants grown in pots of soil in a greenhouse, than from the same soil in the field (De Vries and Tiller, 1978). Therefore

plants which perform poorly in this work, may do so better in the field; however, those which perform well in the pots, should do at least as well in the field, but may not accumulate the same levels of metals as compared to those grown in pots. As field trials are extremely important to real situations, a field experiment was initiated at Prescot site using zeolites P and 4A as soil amendments. The field experiment was carried out to confirm the results of the model pot experiments.

10.2. Experimental

In order to verify the laboratory test results on a pilot field scale, in which the actual soil and environmental conditions are required to give a complete evaluation of the proposal technique when applied to a given hazardous waste site, a field trial was initiated at British Insulated Callender Cables (B.I.C.C.) Rod Rollers Factory, Prescot.

The site: BICC Rod Rollers factory is located on the western outskirts of Prescot, Merseyside (NGR SJ 462917), adjacent to the M57 motorway and the Liverpool-St. Helens railway. The factory was constructed in 1975 on the site of a former refuse tip. The entire site was covered with imported soil which came from excavations associated with the construction of the adjacent motorway. The grasslands which surround the factory were established on this material at the time of construction. The factory produces copper rod via a continuous process, using ingots of 99.99% pure copper as the starting material. Since the start of production, the factory has processed over two million tonnes of copper (T. Hardman, BICC pers. comm.).

At present, the process emits particulate copper (mainly oxides) at a average annual rate of 5.11 mg/m^3 atmosphere, marginally in excess of the suggested limits of $5mg/m^3$ atmosphere. During its period of operation, it is estimated that approximately 26 tonnes of copper have been emitted from the furnace chimney (Inspectorate International, 1993).

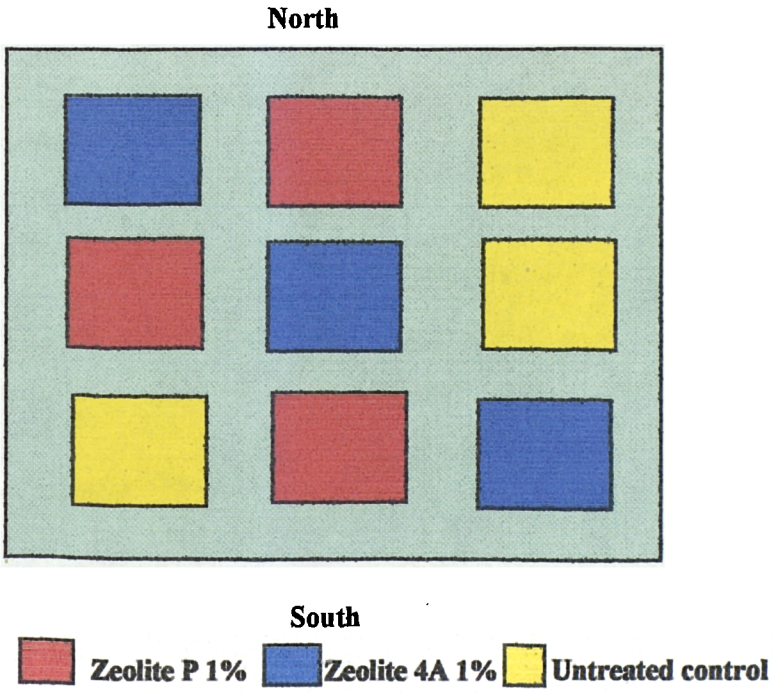
The site suffers from typical symptoms of metal contamination ranging from poor vegetation cover to a lack of diversity in the few species colonising the site. Therefore, zeolites were used as an *in situ* remediation process to reduce the phytotoxicity of the soil.

The success of the remediation was assessed after one year by determining changes in the chemical nature of the soil, and by visually examining the ecology of the trial site, as described below.

The trial was initiated on 13th June 1995 on an area of land 9 x 9 m. The site is

adjacent to the main processing works, and is affected by aerial deposition of copper oxide. The location of the field trial is some distance from where soil samples were collected for the previous experiments described in this work and has a very different pollution history (Lepp *et al.*, in press). Therefore to evaluate substratum phytotoxicity, five top-soil samples (0-20 cm) were taken at random from the experimental area before treatments were applied. The experimental area was divided into three treatments areas (2 x 2 m) and three replicates were randomised over the area. 1 m discard strips were left on all four sides of the plots. The experimental plan is shown in Fig. 10.1. The plots were not fertilised, but they were treated with several passes of a mechanical slitter before the zeolite application. The control plots were set up without any zeolite addition. Any stones or concrete material were previously removed by hand.

Fig. 10.1. Field Trial at Prescot B.I.C.C. initiated on 13th June 1995.



Zeolites P and 4A were chosen for the field experiment for two reasons: first, these two zeolites applied at low concentrations (0.5% and 1%) have been the most efficient amendments for increasing plant biomass and reducing heavy metals bioavailability, and second, their cost of manufacture is considerably cheaper than for zeolite Y (which had to be used at a higher concentration (5%) to produce a comparable effect to that of zeolites P and 4A). The zeolites were applied as a slurry, in water. On six of the nine 2 m x 2 m plots, the

upper 20 cm of the contaminated soil was treated with 1% zeolite P or 4A. The mass of zeolite required was calculated from the density of the soil and volume treated. The three other plots were left as controls. The trial was left for one year, before further analyses to be carried out.

Before and one year after treatment, soil samples were randomly collected from the trial site and dried at 105°C for three days in an air-circulating oven, then ground using an agar mortar and sieved through a 0.5 mm plastic sieve. The pH of the samples was measured following the procedure described in Section 4.2.1.2. The soil samples were extracted with distilled deionised water to determine the water-soluble cadmium, zinc, copper and lead content, as described in Section 4.2.1.2. The total metal content of the soil was determined by digestion with HNO_3 (conc., A.R.) using a microwave digestor (which is described in Section 4.2.1.2.). After extraction and digestion, the total content of heavy metals was estimated using the AAS. Results of triplicate analysis are presented in Tables 10.2. and 10.3.

10.3. Results and Discussion

10.3.1. Chemical Evaluation

Apart from the use of lime as an *in situ* amendment for metal contaminated land, only a limited number of other materials have been investigated, and even fewer field trials have been reported.

Field trials using ion exchange materials to reduce heavy metal uptake by plants or to improve soil fertility have been reported (Wessolek and Fahrenhorst, 1994). The synthetic phillipsite has been used successfully in field experiments to increase the water-holding capacity of different types of soils (Kralova *et al.*, 1994). Field trials with clinoptilolite which was compared to sphagnum peat and sawdust in sand or sandy soils to enhance 'Penncross' creeping bentgrass (*Agrostis palustris*) establishment, moisture retention and C.E.C.s of the resultant mixes, found that all amendments, except 10% and 20% sawdust, resulted in superior establishment compared to unamended sand (Nus and Brawen, 1991).

The addition of 5 to 10% beringite to zinc contaminated land resulted in a reduced heavy metal uptake by plants (*Phaseolus vulgaris*). Growth parameters, enzyme activities and zinc content of the leaves were restored to the control levels (Vangronsveld *et al.*, 1991). Beringite and compost have also been used to remediate the site of a former zinc smelter, strongly contaminated by several nonferrous metals. 18 months after sowing a seed mixture

of selected metal-tolerant plants (*Agrostis capillaris, Festuca rubra*), a healthy and well closed vegetation developed. Addition of 5% beringite to the substratum resulted in a significantly lower metal content in the aerial parts of the plants (Vangronsveld *et al.*, 1995 b).

The initial pH of the soil samples collected at random, from the test plot varied between 3.3 - 3.8, which is very acidic (1: pH = 3.8 (±0.9); 2: pH = 3.3 (±0.4); 3: pH = 3.8 (±0.8); 4: pH = 3.6 (±0.7); 5: pH = 3.6 (±0.6)). After one year, the pH of the untreated plots remained unchanged (C1, 2 and 3), whereas the pH of the amended plots increased above 6 (Table 10.1.).

Table 10.1. pH of the soil samples after one year (n = 5).

Treatment	Sample	pH
Untreated, outside trial plots	R1	3.6 (±0.4)
Untreated, outside trial plots	R2	3.5 (±0.5)
Untreated plot	C1	3.5 (±3.5)
Untreated plot	C2	3.7 (±0.7)
Untreated plot	C3	3.4 (±0.6)
Zeolite P	P1	6.5 (±0.4)
Zeolite P	P2	6.1 (±0.3)
Zeolite P	P3	6.3 (±0.5)
Zeolite 4A	A1	6.0 (±0.3)
Zeolite 4A	A2	6.2 (±0.2)
Zeolite 4A	A3	6.3 (±0.4)

The water- and nitric acid-extractable metal levels before and one year after treatment are given in Tables 10.2. and 10.3. The amount of copper in the soil increased slightly after one year, due to its continued atmospheric deposition from the operational manufacturing processes. This also led to a greater concentration of copper in the water extract, and

consequently more copper is available for plant uptake. No increase in the input of cadmium, zinc and lead was noted. The addition of 1% zeolite P or 4A to Prescot soil considerably decreased the water soluble fraction of all metals.

No differences between the unamended and amended plots were observed for the nitric acid fraction, as this extraction represents the total metal content of the soil, which is not influenced by the addition of synthetic zeolites to the soil.

Table 10.2. Water-extractable and nitric acid-extractable (total) metal concentrations in replicas of five different sites of Prescot soil at the beginning of the experiment (n = 5).

	H_2O-extractable metals ($\mu g\ g^{-1}$)				HNO_3-extractable metals ($\mu g\ g^{-1}$)			
Sample	Cu	Cd	Zn	Pb	Cu	Cd	Zn	Pb
1	9.8 (±0.83)	0.8 (±0.53)	3 (±0.91)	2.4 (±0.05)	1000 (±263)	5 (±2.1)	400 (±32)	500 (±125)
2	10 (±0.72)	0.92 (±0.61)	3.2 (±1.1)	2.3 (±0.02)	1100 (±274)	5.2 (±2.6)	380 (±30)	480 (±111)
3	10.5 (±0.6)	0.71 (±0.48)	2.7 (±0.83)	3 (±0.08)	900 (±288)	5.3 (±2.2)	420 (±35)	500 (±120)
4	10.2 (±0.63)	0.73 (±0.45)	2.6 (±0.87)	2.6 (±0.04)	950 (±250)	6 (±2.7)	360 (±33)	470 (±116)
5	9.5 (±0.57)	0.85 (±0.62)	2.9 (±0.92)	3.1 (±0.08)	1000 (±264)	5.8 (±2.8)	370 (±31)	480 (±113)

10.3.2. Biological Evaluation

To evaluate the durability of the metal-immobilising effect of the soil treatment, the potential phytotoxicity of the treated substrata was tested 12 months after the soil treatment was applied by analysing the colonisation of the treated plots. The plant cover for each plot was assessed visually. Plant metal content was not measured, due to continuing copper deposition on the foliage from the atmosphere.

Table 10.3. Water-extractable and nitric acid-extractable (total) metal concentrations in samples of Prescot soil after one year (n = 5).

		H_2O-extractable metals ($\mu g\ g^{-1}$)				HNO_3-extractable metals ($\mu g\ g^{-1}$)			
Treatment	Sample	Cu	Cd	Zn	Pb	Cu	Cd	Zn	Pb
Untreated, outside trial plots (see Table 10.1.)	Initial Values (Mean)	10 (±0.67)	0.8 (±0.54)	2.9 (±0.93)	2.7 (±0.05)	990 (±268)	5.5 (±2.5)	386 (±32)	486 (±117)
Untreated, outside trial plots (After 1 year)	R1	14.4 (±0.64)	0.7 (±0.52)	3 (±1)	2.9 (±0.06)	1200 (±283)	5.5 (±2.6)	380 (±27.6)	460 (±120)
Untreated, outside trial plots (After 1 year)	R2	13.7 (±0.55)	0.8 (±0.5)	2.5 (±0.72)	2.5 (±0.04)	1300 (±294)	4.9 (±2.2)	350 (±28.8)	470 (±115)
Untreated plot	C1	15 (±0.82)	0.8 (±0.47)	2.8 (±0.8)	2.8 (±0.05)	1200 (±270)	5 (±2.8)	300 (±29.3)	480 (±123)
Untreated plot	C2	14.6 (±0.71)	0.75 (±0.45)	3 (±0.93)	2.6 (±0.03)	1000 (±280)	4.8 (±2)	360 (±29.5)	460 (±110)
Untreated plot	C3	13.8 (±0.68)	0.7 (±0.42)	2.6 (±0.74)	2.5 (±0.07)	1350 (±300)	4.7 (±2.3)	350 (±26.4)	450 (±100)
Zeolite P	P1	3.8 (±0.75)	0.45 (±0.3)	1.3 (±0.53)	2 (±0.02)	1150 (±310)	4.5 (±2)	350 (±25.5)	420 (±112)
Zeolite P	P2	4 (±0.7)	0.42 (±0.24)	1.4 (±0.47)	2.3 (±0.02)	1000 (±290)	5.2 (±2.4)	330 (±27.2)	470 (±115)
Zeolite P	P3	3.5 (±0.73)	0.4 (±0.25)	1.5 (±0.43)	2 (±0.03)	1200 (±270)	4.6 (±2.5)	370 (±26.9)	450 (±118)
Zeolite 4A	A1	3.7 (±1)	0.43 (±0.26)	1.2 (±0.7)	2.2 (±0.01)	1100 (±305)	5.3 (±3)	360 (±28.3)	400 (±130)
Zeolite 4A	A2	4.2 (±0.92)	0.48 (±0.32)	1.6 (±0.64)	2.4 (±0.05)	1250 (±282)	5 (±3.1)	370 (±27.3)	480 (±134)
Zeolite 4A	A3	4.5 (±0.87)	0.5 (±0.28)	1.4 (±0.38)	2.3 (±0.04)	1200 (±271)	4.4 (±2)	320 (±26.5)	460 (±128)

Plate 10.1. Field trial at Prescot site

a. Initially

b. After one year.

At the beginning of the experiment, the plot was colonised only by a few species: *Agrostis sp., Sagina sp., Festuca sp., Rumex acetosa, Carex hirta* and the moss *Pohlia nutans*. After one year, no new species were detected, but there was a considerable increase in numbers of the species already present on the amended plots, as shown in Plates 7.1. (a, b). No comparable increase was observed in the untreated sites.

On the plots treated with zeolite P or 4A, the plants were much greener and healthier than the plants grown on the unamended plots. No signs of chlorosis were detected for the plants grown on the amended plots, whereas the plants grown on the unamended plots were shorter, in smaller numbers and with visible signs of chlorosis, due to the higher amount of metals available for plant uptake on the untreated plots.

The root growth was compared for grasses grown on all plots after one year. A significant enhancement of root growth was noted in the presence of 1% zeolite P (Plate 7.2. b.) and 4A (Plate 7.2 c.) in comparison to the untreated control (Plate 7.2.a.). Also, the addition of synthetic zeolites to Prescot soil initiated the development of new and healthy roots. This effect has considerable importance, as application of synthetic zeolites not only reduce the available metal fraction of the soil, but will also stimulate the growth of new and healthy species.

10.3.3. Conclusions

It is unusual to encounter an industrial process which emits only a single metal in elevated quantities to the immediate environment (Alloway, 1995). In most cases, metals are emitted in varying quantities from metal processing or smelting activities, frequently accompanied by sulphur oxides (Ross, 1994). The site of a Copper Rod-Rolling factory at Prescot, Merseyside, UK in the present investigation represents a rare occasion when the effect of a single metal can be studied in isolation. The site was constructed on land on the outskirts of Prescot with no previous industrial legacy and the surrounding vegetation was established on soil imported from outside the immediate vicinity. Copper is emmited from the process in the form of oxides and the annual emmissions are routinely monitored (Inspectorate International, 1993).

Metals tend to accumulate in topsoils through aerial inputs and by vegetation uptake, assimilation and subsequent litter fall. More studies quantifying the accumulation of metals in topsoils are required to provide evidence for understanding how soil biological processes respond over time.

Plate 7.2. Root Growth in Prescot Soil.

a. Untreated Control.

b. 1% Zeolite P.

c. 1% Zeolite 4A.

By initiating the field trial at Prescot, it was possible to check directly in a landscape environment the results obtained in the laboratory for the three synthetic zeolites: P, 4A and Y, particularly their long-lasting efficiency in heavy metal immobilisation. It is evident that there was a significant decrease in the copper-exchangeable fraction on zeolite-amended soils compared to untreated controls.

There were clear differences in total and water-extractable concentrations between unamended and amended plots. One-way analysis of variance on these data revealed significantly lower ($P < 0.05$) metal bioavailability in zeolite-amended soils as opposed to the untreated controls. For statistical analysis, all data were expressed as zeolite treatment : untreated control ratio and the metal concentrations were statistically analysed following the same procedure described in Section 7.5. For the water-soluble fraction, there were significant differences (from Tukey multiple comparison tests) ($P = 0.000$) between zeolites 4A and P in samples from all plots for copper and cadmium in Prescot soil one year after treatment. No significant differences were noted for water-extractable zinc and lead, but this is not surprisingly, as copper is the main contaminant at Prescot.

The phytotoxicity of the polluted Prescot soil was suppressed using the zeolite amendment, proving an efficient immobilisation of the metals in the substratum over the time

period considered (one year). Nevertheless, the potential phytotoxicity of the treated soil must be followed monitored regularly over a longer period of time.

The results from all the work to date indicate that in terms of the laboratory and column tests, zeolite P and 4A, have been the most suitable candidates for amending land contaminated by non ferrous metals. The synthetic zeolites perform well under field conditions, and it can be anticipated that the further (generative and vegetative) development of the vegetation cover will produce a humus layer, resulting in a supplementary metal-immobilising capacity of the upper soil strata.

By initiating the field trial at Prescot, an assessment about how realistic the effect of synthetic zeolites will be in the field was ascertained. The risk assessment in the actual treatment was evaluated by calculating the long-term efficiency of zeolites P and 4A in the amended plots. Synthetic zeolites are efficient soil amendments, as they decrease the bioavailable fraction of metals by ion-exchange processes. Another advantage of the zeolite application is their long term efficiency. Assuming a constant input of copper to the site, based on the previous year's observation, zeolite P and 4A would be able to ion exchange copper for period of 223 and 211 years respectively at 1% application rate, provided they remained unchanged and *in situ*. Traditional methods of soil amelioration (e.g., liming, application of organic substances) need to be regularly repeated.

Although an important improvement of the root growth in the presence of synthetic zeolites was noted, further work will be necessary for finding suitable methods to stain roots and detecting mycorrhizal presence, as mycorrhizal fungi and vesicular-arbuscular mycorrhiza increase the metal tolerance of the host plant by restricting passage of metals to the shoots, therefore providing an effective exclusion barrier.

Ectomycorrhizal fungi have a role in the survival of plants in metal-contaminated soils by enabling avoidance of the metal stress. The ameliorating influence of the ectomycorrhizal fungi on metal toxicity is linked with compatibility of the fungal strain with the host. Colonisation by fungal mycelium lead to metal adsorption on hyphal surfaces. As a result, less metal will be taken up by the host. Further research is required into the adaptations of the fungi to metal-contaminated environments and the role of the symbiosis on their survival. In addition, there is a need for research into the toxicity of heavy metals to mycorrhiza and their role in ameliorating plant metal uptake in contaminated environments. The presence of infection in natural environments could partially explain why lower concentrations of metals are generally found in plants in the field by comparison with those grown in pots in controlled environments.

Ecosystem development is an evolutionary process, and it is consequently unrealistic to expect a properly functioning ecosystem immediately following reclamation. Nevertheless, the revegetated area should be at least equal to that which existed initially and be capable of stabilising the soil surface from erosion. The monitoring of habitats would provide an index of the ecological performance of reclaimed land, as well as assisting in the assessment of landscape quality (Tomlinson, 1984).

The need for long-term research in the field of ecology, particularly applied ecology and ecological toxicology, is vital to understand fully how a system functions following chronic long-term contaminations.

CHAPTER 11.

GENERAL CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK.

This work was undertaken to evaluate the potential use of synthetic zeolites as amendments for polluted soils. The capacity of synthetic zeolites to fix heavy metals in polluted soils was evaluated by laboratory, greenhouse and field trial experiments.

The aim of the study was to investigate synthetic zeolites with respect to their heavy metal absorption in contaminated soils, and namely: (1) to reveal zeolite capabilities of absorbing and fixing heavy metals; (2) to trace changes of heavy metal content in vegetables as a result of zeolite effects; and (3) to show zeolite effects on the speciation of heavy metals in amended soils.

Modelling the fate of metals in ecosystems after amending polluted soils with synthetic zeolites has been used to understand, predict and simulate the fate and distribution of metals in soils and in plants, but also has taken into account food chain contamination in soil-plant systems.

The extent of heavy metal pollution of plant products grown in an industrially polluted zone varies widely. The unequal content of heavy metals in vegetable crops depends on local industrial and geochemical conditions, on the type of crop (its ability to limit the inflow of excess ions into edible parts of the plant), and on the chemical characteristics the priority heavy metals have in accumulating in the plants.

The application of zeolites in reducing soil phytotoxicity is suggested in two areas of major concern: 1) for safer food production in slightly polluted soils, and 2) in highly polluted areas which are devoid of vegetation, such as in the vicinity of metal smelters, as a reclamation policy to encourage the development of a vegetation cover, with the main objective of reducing pollution dispersion by wind erosion and groundwater percolation (Vangronsveld and Clijsters, 1992).

The efficiency of synthetic zeolites as potential amendments for *in situ* land reclamation was investigated gradually, by analysing their effect upon each link of the food chain. The zeolite characteristics were identified by TGA, SEM and XRD techniques. The synthetic zeolites were then exchanged with different metals in solution and binary ion-exchange isotherms were determined. This allowed a first estimation of their ability to trap heavy metals by ion-exchange processes. The overall efficiency of zeolites for ion-exchange was: 4A > P > Y (copper (%): 64 > 63 > 59; cadmium (%): 68 > 55 > 42; zinc (%): 70 > 67

> 65 and lead (%): 50 > 45 > 44) (Table 3.10.). The possibility of reclamation of polluted soils, strongly contaminated by different heavy metals, was studied by amending the soils with three different synthetic zeolites: P, 4A and Y. The efficiency of three synthetic zeolites for reducing metal bioavailability was tested on three contaminated soils: Trelogan, Prescot and Gateacre. These three soils were amended with each zeolite in three different concentrations (0.5%, 1% and 5%), whereas untreated soil samples served as controls.

Correlations between extractable fractions and uptake by test plants have been used to estimate bioavailability of soil metals. The complexity of soil metal reactions and transformations is the reason why it is so difficult to predict soil metal bioavailability, mobility and retention (Ross, 1994). It is important to be able to identify the forms of metals in the soil, especially in the soil solution, in order to more fully understand the dynamics of the metal in agricultural and natural ecosystems.

A sequential extraction procedure on zeolite-amended soils indicated a metal redistribution after incubating the soils with synthetic zeolites, namely a decrease in the exchangeable metal fraction and an increase in the residual fraction for all three zeolites. The mobility, bioavailability, and potential phytotoxicity of copper, cadmium, lead and zinc were investigated using soil extractions (i.e. water, ammonium acetate, hydrogen peroxide and nitric acid). The bioavailable fraction of metals decreased significantly after adding the zeolites to the soils. As a general trend, increasing zeolite concentration from 0.5% up to 5% resulted in more exchangeable metals being trapped in the zeolite cages. The highest efficiency was shown by zeolite P, which decreased the ammonium acetate-extractable metals in amended soils by 60% for Trelogan, 72.4% for Prescot and 65.6% for Gateacre (Fig. 5.2.).

In order to estimate how realistic the results of the sequential extraction experiments may be in relation to soils, the potential beneficial effect of these amendments on metal leaching from the contaminated soils was estimated. The ion exchange properties of the zeolites can be used to retain selected ions in the soil. The main action of an effective sorption additive is to enhance the capacity of the soil and retain the contaminant to minimise leaching. Soil solution studies indicated the potential of using synthetic zeolites for reducing metal leaching from contaminated soils. Semi-field simulations of percolation showed that the beneficial effect of synthetic zeolites was marked: the amount of percolating metals was reduced with more than 40% for zeolite Y and over 50% for the other two zeolites. The relatively high mobility of cadmium and zinc (through the soil profile) were evident from the distribution of these metals in the ammonium acetate and water soluble fractions of unamended soils. A smaller amount of copper and lead were present in these leachates and

the concentrations of all four elements studied were much less in the presence of synthetic zeolites. The increased amount of zeolites (0.5, 1 and 5%) and the type of zeolite (P, 4A or Y) influenced the values.

Column experiments (Chapter 6) indicated that the metal-exchanged zeolites are stable over a period of several months, as indicated by continually low metal concentrations in the eluent. The addition of synthetic zeolites to contaminated soils is very effective as a remedial treatment, even at low rates of application (0.5% and 1% by weight). After adding synthetic zeolites to the contaminated soils, the bioavailable fraction of metals decreased by over 40% for zeolites P and 4A and by 32% for zeolite Y in Trelogan soil, by more than 52% for zeolites P and 4A and by 45% for zeolite Y in Prescot soil, and by 47% for zeolite P, 51% for zeolite 4A and only by 39% for zeolite Y for Gateacre soil (Tables 6.3. - 6.6.).

The effect of the three synthetic zeolites: P, 4A and Y upon the second link of the food chain, the plants, was evaluated in greenhouse experiments with mono- and dicotyledonous plants.

The three soils under study are contaminated with heavy metals: Trelogan soil contains 80 $\mu g.g^{-1}$ cadmium, 17 700 $\mu g.g^{-1}$ zinc and 10 300 $\mu g.g^{-1}$ lead (Table 4.4.), which are far above the critical concentration of these metals in soils: 8 $\mu g.g^{-1}$ for cadmium and 400 $\mu g.g^{-1}$ for zinc or lead (Table 4.5.). Prescot soil is highly contaminated with copper (1100 $\mu g.g^{-1}$), concentration far in excess from the critical permissible copper level in soils (125 $\mu g.g^{-1}$). Gateacre soil has a high concentration of cadmium (25 $\mu g.g^{-1}$), compared to normal soil (8 $\mu g.g^{-1}$), and also lead (575 $\mu g.g^{-1}$), concentration above the maximum permissible level (400 $\mu g.g^{-1}$) (Alloway, 1995).

Greenhouse and field investigations were conducted to determine the effect of incorporating synthetic zeolites into polluted soils on plant yield and metal concentration. Glasshouse experiments were designed to study the direct effect of synthetic zeolites 4A, P and Y on the dry matter yield of four test plants: ryegrass, sunflower, maize and willows. The high levels of contaminant metals in the plant tissue of trial plants grown on the three contaminated soils reached phytotoxic concentrations in nearly all cases. The tissue concentration of all the metals studied showed a significant reduction in the presence of synthetic zeolites in the roots and leaves of the test plants in zeolite-amended soils.

Copper: The normal copper range in plants is 5-20 $\mu g.g^{-1}$, and the critical range is 20-100 $\mu g.g^{-1}$ (Table 4.5.). For the foliage of ryegrass, sunflower and maize grown in Gateacre soil, the copper concentrations were below the upper copper critical range (100

$\mu g.g^{-1}$) for the unamended soil and in the normal range (5-20 $\mu g.g^{-1}$) for the zeolite-amended Gateacre soil (Fig. 7.2., 7.15. and 7.16.). For sunflower and maize plants grown on amended or unamended Prescot soil, the copper concentrations were phytotoxic, as they were above the critical limit. The same results were obtained for willow shoots in Prescot soil (Fig. 7.9.-7.12. and 7.41.). The copper concentration for the ryegrass grown on amended Prescot soil was just under the critical upper limit. For the roots of sunflower and maize grown on Gateacre soil, the copper concentrations were under the upper critical limit for untreated and treated soil, but the presence of synthetic zeolites decreased copper concentrations to lower values than the untreated control. The same results were obtained for willow shoots in Gateacre soil and for willow leaves in Prescot and Gateacre soil (Fig. 7.1., 7.13., 7.14. and 7.42.-7.44.).

Cadmium: The normal cadmium range in plants is 0.1-2.4 $\mu g.g^{-1}$, and the critical range is 5-30 $\mu g.g^{-1}$ (Alloway, 1995). Amending Prescot soil with synthetic zeolites resulted in a decrease of cadmium concentrations in ryegrass, sunflower and maize plants. Cadmium levels were in the normal range, therefore not phytotoxic for the plants (Fig. 7.5. and 7.17.-7.20.). For zeolite-amended Gateacre soil, the cadmium concentrations were well below the upper critical limit. The addition of synthetic zeolites to this soil caused a decrease of phytotoxicity in the test plants. The same results were noted for willow in both amended soils. Cadmium concentrations in the unamended Gateacre soil were under the upper critical limit (Fig. 7.6., 7.21.-7.24. and 7.45.-7.48.).

Zinc: For zinc, the upper limit is equal for normal and critical range in plants, namely 400 $\mu g.g^{-1}$ (Table 4.5.). For sunflower plants and maize roots grown on Prescot soil, the synthetic zeolites decreased the zinc concentrations below 400 $\mu g.g^{-1}$. Zinc concentrations in the unamended Prescot and Gateacre soil for ryegrass, sunflower and willow and also for maize foliage in Prescot soil were in the normal range and the addition of zeolites to these soils resulted in an important decrease in zinc concentrations towards the lower normal limit in plants.

Lead: The normal lead range in plants is 0.2-20 $\mu g.g^{-1}$, and the critical range is 30-300 $\mu g.g^{-1}$ (Alloway, 1995). Addition of synthetic zeolites to Prescot and Gateacre soils resulted in a decrease in lead content of the test plants under the upper critical limit (300 $\mu g.g^{-1}$) (Fig. 7.3., 7.4., 7.33-7.40. and 7.53.-7.56.).

Amending polluted soils with synthetic zeolites decreased the total metal fraction in plants to less phytotoxic levels than the untreated controls, but the metal

concentrations in the plant tissues remained above the upper critical limit of metals in plants, which are: 100 $\mu g.g^{-1}$ for copper, 30 $\mu g.g^{-1}$ for cadmium, 400 $\mu g.g^{-1}$ for zinc and 300 $\mu g.g^{-1}$ for lead (Table 4.5.) (Alloway, 1995).

It is not certain whether the toxicity symptoms produced in the plant were solely due to the excessive levels of heavy metals in the plant tissue or that the toxicity was associated with ionic imbalance involving other essential trace metals. The measured levels of contaminant metals in tissues are indicators of potential toxicity but the development of toxicity may be associated more with the localisation and chemical forms of the metals at the cellular level. Of the four metals studied, zinc and cadmium were more concentrated in the plant shoots and copper and lead in the plant roots. Comparing these observations with the chemical forms of the metals from the sequential extraction experiments (Chapter 5), the mobility of cadmium and zinc were found to be higher than copper and lead within the soil-plant system studied.

Metal accumulation in food crops at phytotoxic levels is a great cause of concern due to the risk of increased dietary exposure in consumers. The determination of the heavy metals in tissue of food plants is an important factor in determining their accumulation in the human body. Addition of synthetic zeolites to contaminated soils decreased the metal concentrations in the plant tissues under the phytotoxic concentrations. By applying synthetic zeolites to less contaminated soils, the metal concentrations in the plant tissues could decrease below the critical range of metals in plants, therefore improving the quality of the crops.

ANOVA and Tukey's Multiple Comparison Test were used to determine significant differences ($P < 0.05$) between short- and long-term treatments regarding zeolite type and concentration, plant and soil heavy metal concentrations, and plant species productivity. For all three zeolites, there was a clear trend towards decreased metal bioavailability in samples taken from the sewage-sludge and copper refinery sites, even though some of the analysed metals did not occur in elevated amounts at all locations.

Application of synthetic zeolites to polluted soils results in a sharp decrease in the concentration of bioavailable heavy metals. The reduced concentrations of mobile forms of heavy metals in the amended soils had a positive effect on plant growth and heavy metal content in the plant parts (roots, stems and leaves).

The effect of synthetic zeolites on the total metal contents depended upon their effects on both the metal concentrations and dry matter yields of the test plants. The total

metal uptake by plants was calculated by multiplying the metal concentration with dry matter yields of the plant species for each pot (Appendix 4).

Ryegrass: For ryegrass in Prescot soil, zeolite P at 5% decreased the total metal content compared to untreated control. All zeolites at all concentrations decreased the total zinc and lead uptake below that of compost. The total uptake of copper, cadmium and zinc was lower for Gateacre soil amended with zeolite P and 4A compared to untreated control. The same result was obtained for lead when zeolite P was applied to Gateacre soil. For zinc and lead, the total uptake was lower when Gateacre soil was amended with zeolite Y at 0.5% (1 mg/pot) and 5% (1.35 mg/pot). All zeolites at all concentrations decreased the total copper uptake below that of compost (Appendix 4 - Table 4.1.).

Addition of synthetic zeolites to Prescot soil increased cadmium uptake by ryegrass compared to the untreated control and compost, therefore increasing the risk of metal transfers in the food chain. Amending Gateacre soil with zeolite Y produced a similar effect (Appendix 4 - Table 4.1.).

Sunflower: For sunflower in Prescot soil (Appendix 4 - Table 4.2.), zeolites P and Y at all concentrations and 1% zeolite 4A decreased the total copper and zinc uptake below that of compost for the roots. The total lead uptake was lower when zeolites were applied to Prescot soil compared to the lead uptake for the sunflower plants grown on compost soil (Appendix 4 - Table 4.2.). All zeolites decreased the total metal uptake below that of compost or untreated control for copper, cadmium and zinc in the sunflower plants grown on Gateacre soil. Zeolite Y decreased the total lead uptake below that of compost for the sunflower foliage in Gateacre soil. For roots, the same zeolite gave identical results only when it was applied at lower concentrations (0.5% and 1%) (Appendix 4 - Table 4.3.).

Zeolites increased the total copper uptake in the sunflower roots and also the total cadmium uptake above that of compost for the sunflower plants grown on Prescot soil (Appendix 4 - Table 4.2.). The sunflower plants did not survive in unamended Prescot soil. All zeolites increased cadmium uptake above the compost level for the sunflower foliage in Gateacre soil (Appendix 4 - Table 4.3.).

Maize: For maize roots in amended Prescot soil, the total copper and lead uptake were below that of untreated control or compost. In addition, zeolite Y decreased the total cadmium and zinc uptake below that of compost and the total metal uptake was lower than for untreated control when zeolite Y was applied at the lowest rate (0.5%)

(Appendix 4 - Table 4.4.). For maize plants in Gateacre soil, all zeolites at different concentrations decreased the total metal uptake below that of untreated control. The copper uptake was lower in amended Gateacre soil compared to compost. Zeolite P at 1% and 5% decreased the total zinc (0.17 mg/pot for 1% zeolite and 0.12 mg/pot for 5%) and lead (0.1 mg/pot for both concentrations) uptake below that of compost (0.3 mg/pot zinc and 0.21 mg/pot lead) for maize roots (Appendix 4 - Table 4.5.).

Zeolites P and 4A increased the total uptake of all metals for maize roots in Prescot soil, as they increased the dry matter yields. Both zeolites gave the same results also for copper and cadmium uptake in the foliage (Appendix 4 - Table 4.4.). The total cadmium uptake was higher than for compost for the maize plants grown on amended Gateacre soil (Appendix 4 - Table 4.5.).

Willow: The total metal uptake was lower than for untreated control for willow (shoots) when grown on zeolite amended Prescot soil, and also copper uptake was decreased for leaves in amended Prescot soil. For the leaves of the willow grown on Prescot soil, zeolite Y decreased the total cadmium, zinc and lead uptake compared to untreated control (Appendix 4 - Table 4.6.). 0.5% zeolite P and 4A decreased the total metal uptake compared to the untreated control for the willow (shoots) grown on Gateacre soil. The same zeolites at the same concentrations decreased zinc uptake (0.13 mg/pot for shoots and 0.19 mg/pot with zeolite P and 0.2 mg/pot with zeolite 4A for leaves) compared to untreated control (0.23 mg/pot for shoots and 0.25 mg/pot for leaves) and also copper uptake when zeolite P was applied (0.054 mg/pot with zeolite P compared to 0.08 mg/pot for untreated control for shoots and 0.12 mg/pot with zeolite P compared to 0.16 mg/pot for untreated control for leaves) and cadmium uptake (0.044 mg/pot with zeolite and 0.054 mg/pot without zeolite for leaves) with zeolite 4A at the lowest concentration (0.5%). A higher concentration (1%) of zeolite P also decreased the total copper, cadmium and zinc uptake below that of untreated control for willow (leaves) in Gateacre soil, whereas the addition of zeolite 4A decreased copper, cadmium and zinc uptake for willow leaves and also zinc uptake in the shoots of willow grown on Gateacre soil. Zeolite Y had to be applied at the highest concentration (5%) in order to decrease copper and zinc uptake for willow leaves in Gateacre soil (Appendix 4 - Table 4.7.).

Zeolites P and 4A increased zinc uptake for willow (leaves) in Prescot soil above that of untreated control (0.42 mg/pot) and far above that of compost (0.13 mg/pot) (Appendix 4 - Table 4.6.). The willows did not survive when Gateacre soil was amended

with zeolite Y at lower concentrations (0.5% and 1%). 5% zeolite Y increased total metal uptake for shoots and cadmium (0.055 mg/pot) and lead (0.57 mg/pot) uptake for the leaves of the willow grown in Gateacre soil (Appendix 4 - Table 4.7.).

These results demonstrate the potential of synthetic zeolites as amendments for polluted soils and an assessment of the overall remedial capacity of metal-immobilising substances. The addition of synthetic zeolites to the polluted soils decreased levels of heavy metals in plant tissues to less phytotoxic concentrations compared to untreated controls and increased growth considerably. A decrease in plant metal concentration, following a strong growth response to applied synthetic zeolites, was clearly observed.

The synthetic zeolites decreased the bioavailable fraction of metals by a double effect: ion-exchange processes and pH increase. The lower plant accumulation of heavy metals when grown in polluted soils amended with synthetic zeolites can be attributed to the limited number of metal cations available for retention by the negative sites of the plant tissues.

Other soil amendments decrease the mobility of metals in soils by a pH increase, therefore different amendments (lime and compost) were tested to compare their ability to immobilise heavy metals in contaminated soils.

Addition of zeolite 4A to compost soil increased total uptake for zinc and lead compared to untreated compost (Table 8.1.). Amending Prescot soil with zeolite 4A decreased total uptake for all four metals in the sunflower plants (Tables 8.1. and 8.2.) compared to lime, and, most significant, the total uptake was lower than for untreated compost for cadmium, zinc and lead.

Increasing the amount of fertiliser increased total metal uptake for cadmium, zinc and lead for ryegrass grown on treated and untreated Trelogan soil. The total copper uptake was lower than for compost (0.012 mg/pot for Trelogan amended with zeolite P at 0.5% compared to 0.062 mg/pot for compost), but the metal uptake increased proportionally with the increase of fertiliser rates (Table 9.2.).

The most effective proved to be synthetic zeolites, trapping and holding heavy metals by a process of ion-exchange. Consequently, metal solubility, mobility and toxicity are greatly reduced resulting in diminished leaching by rain or groundwater.

Preliminary pot trials provided an indicator which would subsequently assist in planning field trials, as considerable problems are inevitably encountered when the results of laboratory investigations are extrapolated to the 'real world'. A field trial was initiated at B.I.C.C. Prescot, in order to check directly in a landscape environment the results obtained in the laboratory for the synthetic zeolites: P and 4A, particularly their long-

lasting efficiency in heavy metal immobilisation. Zeolites P and 4A applied at 1% level proved to be an effective treatment for the remediation of the contaminated site, as indicated by improved plant growth and low metal concentrations in the water soluble fraction of the soil.

The results of this study are in agreement with previous results obtained by other researchers (Breck, 1974; Gworek and Borowiak, 1991; Gworek, 1992) in model, pot (greenhouse), and field experiments concerning the addition of synthetic zeolites into polluted soils and the binding of heavy metals by natural and synthetic zeolites.

The data obtained from laboratory experiments and greenhouse trials allowed a more comprehensive understanding of heavy metal immobilisation by synthetic zeolites due to ion-exchange mechanisms.

A solution to the problem of land reclamation can be an enhanced immobilisation of the metals through the addition of metal-immobilising soil additives. This was demonstrated by the significant decrease in the bioavailable fraction of metals after adding synthetic zeolites to the contaminated soils. This study has shown that, on metal polluted soils, the use of synthetic zeolites was an efficient reclamation technique, giving reasonable prospects of long-term success on these sites.

Further Directions

This work has evaluated the potential usefulness of synthetic zeolites as amendments for polluted soils. The results suggest that benefits certainly exist, however further work is necessary to demonstrate the economic value of synthetic zeolites in a soil fertility management system. The suitability of zeolites as an amendment to soils in terms of an economically viable soil fertility program will depend on their interaction with nutrients applied as conventional fertilisers.

Synthetic zeolites exhibited a promising potential to reduce heavy metals transfer from soil to soil solution and their entry into the food chain via plant uptake. These materials are also promising for *in situ* remediation technologies geared to restore heavy metal-contaminated soils. However, little is known about the kinetics and persistence of these additives under field conditions. In particular, the degree of mixing between treatment chemicals and contaminated soil to achieve complete immobilisation and the required dosages should be evaluated.

Work planned for future investigations includes a more realistic assessment of the length of time that each amendment will remain viable, and how long conditions need to be ameliorated for vegetation to become established and self sufficient. Monitoring test sites when established will follow the success of each amendment in the field.

The optimum levels of application must still be determined for individual soil types and species, as must the frequency of application, the optimum particle size, and the nature of possible chemical pretreatments. Future work leave open possibilities for continuing to monitor ecosystem structural and functional changes following zeolite application, looking at sites with low metal contamination, amending them with various natural and synthetic zeolites.

Relatively few studies have continued to monitor crop yields or changes in soil physical and chemical properties over an extended period of time with zeolite applications. Past research indicates that bioavailability and leaching of nutrients are influenced by zeolites, but exact relationships have not been documented for essential plant nutrients, heavy metals, or various soil types (clay, loam, sand, etc.). Long-term studies (5 years or longer) are needed to determine plant uptake of elements from contaminated soils that have been amended with synthetic zeolites.

The resistance of synthetic zeolites to chemical weathering, microbial degradation, low pH, or varying pE is unknown, which further suggests the need for more comprehensive long-term investigations.

Cost-efficient and cost-effective methods for applying synthetic zeolites to soil have not been investigated, nor have the various methods of applying and incorporating zeolites been adequately compared (presowing to seedbed; addition at seedling stage; multi-application throughout growth, compared to an initial, single application). Questions still arise as to whether they should be surface applied, trenched, banded, plowed under, disked in, or applied as a mulch.

Overall, the potential for using synthetic zeolites for the application discussed in this study is promising. However, a number of areas require additional research before synthetic zeolites can be used safely and reliably for all their potential uses. The areas of research include the following: (i) The optimum application parameters for use of synthetic zeolites need to be defined. This involves study of the rate, time, method and frequency of application to specific soil types. (ii) Marketing studies with farmers and local authorities to assess their acceptance of synthetic zeolites as soil conditioners should be conducted. (iii) Determination of the best method(s) of applying synthetic zeolites to land is needed.

The numerous possibilities which exist to utilise the unique chemical and physical properties of synthetic zeolites will surely receive even greater attention in the future.

Finally, there is an urgent need for standardised metal toxicity assessments in contaminated soils and for the development and long-term monitoring of *in situ* contaminated soil treatments for land and habitat restorations.

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Appendix 1

Table 1.1. Amount of copper (ppm) extracted sequentially from the amended soils (Mean of three replicate samples).

	Trelogan				Prescot				Gateacre			
Treatment	NH_4OAc	H_2O_2	HNO_3	Total	NH_4OAc	H_2O_2	HNO_3	Total	NH_4OAc	H_2O_2	HNO_3	Total
No zeolite	16 (±0.5)	35 (±0.5)	34 (±1)	85	75 (±5)	410 (±10)	500 (±0)	985	17 (±1.7)	23 (±2.6)	30 (±2)	70
P 0.5%	4.5 (±0.5)	30 (±5)	47.5 (±1)	82	46 (±6)	335 (±15)	437.5 (±33)	818.5	10 (±0)	20 (±0)	40 (±2.6)	70
P 1%	4 (±0)	16 (±1)	50 (±1)	70	38 (±3.5)	325 (±8.7)	528.5 (±28.5)	891.5	8 (±2)	18 (±1)	45 (±0)	71
P 5%	3.5 (±0.5)	18 (±1)	58.5 (±0)	80	35 (±5)	290 (±17.3)	545 (±42.7)	870	7 (±1.3)	15.5 (±1.3)	50 (±2)	72.5
A 0.5%	8(±0.5)	18 (±1)	48.5 (±0.5)	74.5	45 (±5)	350 (±43.6)	480 (±20)	875	11.5 (±1.3)	18.5 (±1.3)	41 (±1.7)	71
A 1%	7 (±0.5)	16 (±0.5)	50 (±0)	73	40 (±10)	320 (±26.5)	490 (±36)	850	8.5 (±0.5)	17 (±1.7)	45 (±3)	70.5
A 5%	4(±0)	14 (±1)	56 (±0.5)	74	30 (±0)	290 (±36)	565 (±44.5)	885	7 (±0.9)	14 (±1.7)	50 (±0)	71
Y 0.5%	8.5 (±0.5)	20 (±1)	48 (±0.5)	76.5	50 (±5)	380 (±20)	455 (±18)	885	14 (±2.6)	22 (±1.7)	33 (±2.6)	69
Y 1%	8 (±0.5)	18 (±0.5)	55 (±1)	81	45 (±5)	310 (±10)	538 (±10.4)	893	12.5 (±0.5)	17.5 (±0.5)	42 (±1.7)	72
Y 5%	7 (±0.5)	16 (±0.5)	57.5 (±0.5)	80.5	40 (±5)	315 (±13.3)	557.5 (±7.5)	912.5	10 (±0)	16 (±1)	44 (±1.7)	70

Table 1.2. Amount of cadmium (ppm) extracted sequentially from the amended soils (Mean of three replicate samples).

	Trelogan				Prescot				Gateacre			
Treatment	NH_4OAc	H_2O_2	HNO_3	Total	NH_4OAc	H_2O_2	HNO_3	Total	NH_4OAc	H_2O_2	HNO_3	Total
No zeolite	37.5 (±2.3)	15.5 (±0.5)	20 (±0)	73	1.2 (±0.3)	0.6 (±0.05)	2.55 (±0.1)	4.35	9.5 (±0.5)	3.5 (±0.5)	10 (0.87)	23
P 0.5%	15 (±1)	15 (±1)	42.5 (±2.5)	72.5	0.6 (±0.1)	0.55 (±0.05)	3 (±0)	4.15	9 (±0.5)	3 (±0)	11 (±1)	23
P 1%	14.5 (±0.5)	13 (±1)	45 (±2)	72.5	0.5 (±0.1)	0.4 (±0)	3.5 (±0.3)	4.4	8.5 (±0.5)	2.5 (±0.44)	12 (±1.7)	23.5
P 5%	13 (±1.3)	12.5 (±0.5)	46 (±2)	71.5	0.4 (±0.1)	0.35 (±0.05)	3.8 (±0.2)	4.55	7 (±0.5)	2 (±0.44)	15 (±1.7)	24.2
A 0.5%	14.5 (±0.5)	14 (±0.9)	42 (±1.7)	70.5	0.5 (±0)	0.5 (±0.05)	3.2 (±0.35)	4.2	8.5 (±0.5)	2.8 (±0.2)	10 (±0)	21
A 1%	13.5 (±0.5)	13 (±0.9)	44 (±1.7)	70.5	0.4 (±0.1)	0.4 (±0.1)	3.8 (±0.36)	4.6	8 (±0.5)	2.5 (±0.5)	11.5 (±1.3)	22
A 5%	12.5 (±0.9)	12 (±2)	47 (±2.6)	71.5	0.2 (±0)	0.35 (±0.03)	4.2 (±0.36)	4.75	7 (±0.5)	2 (±0)	13.5 (±0.5)	23
Y 0.5%	16 (±1.7)	14 (±0.5)	40 (±0)	70	0.6 (±0.1)	0.56 (±0.04)	2.8 (±0.2)	4	9 (±0.5)	3 (±0)	10 (±0)	22.3
Y 1%	15 (±0)	14.5 (±0.9)	43 (±2.6)	72.5	0.4 (±0.1)	0.5 (±0.1)	3.2 (±0.36)	4.1	8.5 (±0.5)	2.8 (±0.35)	11 (±1.7)	22.5
Y 5%	14 (±2)	13 (±1.7)	45 (±1.7)	72	0.3 (±0)	0.4 (±0.05)	4 (±0)	4.7	8 (±0.5)	2.5 (±0.2)	13 (±0.5)	23.6

Table 1.3. Amount of zinc (ppm) extracted sequentially from the amended soils (Mean of three replicate samples).

	Trelogan				Prescot				Gateacre			
Treatment	NH_4OAc	H_2O_2	HNO_3	Total	NH_4OAc	H_2O_2	HNO_3	Total	NH_4OAc	H_2O_2	HNO_3	Total
No zeolite	8500 (±500)	4850 (±132)	3500 (±100)	16850	255 (±5)	60 (±5)	55 (±5)	370	120 (±10)	85.26 (±0.06)	62 (±3)	267.26
P 0.5%	4700 (±100)	2600 (±100)	9700 (±300)	17000	100 (±10)	55 (±5)	200 (±7.6)	355	50 (±10)	43 (±3.6)	170 (±0)	263
P 1%	3840 (±144)	1800 (±557)	11300 (±300)	16940	70 (±0)	50 (±5)	250 (±10)	370	35 (±5)	41 (±1.7)	175 (±10)	251
P 5%	3300 (±100)	800 (±1000	13000 (±500)	17100	40 (±5)	48 (±0)	270 (±5)	358	30 (±5)	40 (±2)	190 (±10)	260
A 0.5%	4900 (±153)	3500 (±0)	8500 (±500)	16900	110 (±5)	50 (±5)	200 (±7.6)	360	55 (±10)	42 (±0)	175 (±5)	272
A 1%	4200 (±100)	2500 (±100)	10000 (±0)	16700	75 (±0)	43 (±3)	250 (±10)	368	42 (±2)	40 (±2)	185 (±0)	267
A 5%	3400 (±100)	1700 (±100)	11800 (±200)	16900	50 (±10)	37 (±2)	280 (±5)	367	40 (±5)	37 (±3)	190 (±10)	257
Y 0.5%	5900 (±100)	4600 (±100)	6400 (±100)	16900	125 (±5)	63 (±5)	180 (±5)	368	70 (±5)	44 (±2)	170 (±5)	284
Y 1%	5600 (±100)	4400 (±100)	6600 (±400)	16600	110 (±10)	45 (±5)	200 (±5)	355	65 (±5)	42 (±2)	172 (±5)	279
Y 5%	4200 (±200)	4200 (±200)	8000 (±500)	16400	55 (±5)	40 (±0)	270 (±5)	365	38 (±0)	40 (±2)	177 (±7)	255

Table 1.4. Amount of lead (ppm) extracted sequentially from the amended soils (Mean of three replicate samples).

	Trelogan				Prescot				Gateacre			
Treatment	NH_4OAc	H_2O_2	HNO_3	Total	NH_4OAc	H_2O_2	HNO_3	Total	NH_4OAc	H_2O_2	HNO_3	Total
No zeolite	2000 (±0)	5200 (±200)	3000 (±0)	10200	125 (±10)	240 (±10)	140 (±10)	505	124 (±6)	270 (±10)	171 (±0)	565
P 0.5%	1800 (±200)	5000 (±0)	3400 (±100)	10200	95 (±5)	200 (±10)	200 (±10)	495	110 (±5)	255 (±10)	200 (±10)	565
P 1%	1650 (±50)	4900 (±300)	3500 (±100)	10050	90 (±0)	195 (±0)	210 (±10)	495	95 (±10)	240 (±10)	225 (±10)	560
P 5%	1000 (±87)	4700 (±100)	4500 (±100)	10200	85 (±5)	190 (±10)	225 (±10)	500	68 (±3)	230 (±0)	275 (±10)	573
A 0.5%	1700 (±100)	4300 (±100)	4000 (±0)	10000	94 (±4)	210 (±10)	200 (±10)	504	115 (±5)	250 (±0)	200 (±5)	565
A 1%	1500 (±100)	4200 (±200)	4600 (±50)	10300	93 (±0)	200 (±10)	210 (±5)	503	85 (±0)	230 (±5)	250 (±10)	565
A 5%	1250 (±50)	4000 (±200)	500 (±200)	10250	90 (±5)	180 (±10)	230 (±5)	500	62.5 (±0)	220 (±10)	285 (±10)	568
Y 0.5%	1100 (±100)	5000 (±200)	4000 (±100)	10100	100 (±0)	195 (±10)	210 (±15)	505	100 (±10)	240 (±10)	225 (±20)	565
Y 1%	1000 (±0)	4800 (±200)	4500 (±100)	10300	90 (±5)	190 (±10)	220 (±0)	500	90 (±5)	230 (±15)	250 (±0)	570
Y 5%	900 (±100)	4600 (±100)	4800 (±200)	10300	80 (±10)	180 (±10)	240 (±5)	500	75 (±5)	225 (±10)	275 (±10)	575

Appendix 2

Table 2.1. pH of Trelogan samples (Mean of three replicates).

	Soil pH				pH of leachate			
Sample	Initially	After one month	After two months	After three months	Initially	After one month	After two months	After three months
Unamended	**7.2 (±0.4)**	**7.7 (±0.6)**	**7.6 (±0.4)**	**7.5 (±0.2)**	**7.4 (±0.2)**	**7.8 (±0.1)**	**7.5 (±0.1)**	**7.6 (±0.2)**
P 0.5%	10.2 (±0.3)	10.4 (±0.5)	10.6 (±0.5)	10.5 (±0.3)	9.0 (±0.5)	9.2 (±0.3)	8.7 (±0.2)	8.5 (±0.3)
P 1%	10.4 (±0.5)	10.8 (±0.7)	10.3 (±0.3)	10.6 (±0.4)	9.4 (±0.3)	9.9 (±0.3)	9.7 (±0.3)	9.0 (±0.2)
P 5%	11.3 (±0.4)	11.4 (±0.4)	11.5 (±0.4)	11.0 (±0.4)	10.0 (±0.2)	10.2 (±0.6)	9.8 (±0.2)	10.0 (±0.3)
A 0.5%	10.2 (±0.3)	10.4 (±0.5)	10.5 (±0.2)	10.8 (±0.3)	7.6 (±0.7)	9.0 (±0.3)	8.7 (±0.3)	8.5 (±0.2)
A 1%	10.6 (±0.5)	10.8 (±0.4)	11.0 (±0.6)	10.5 (±0.3)	10.0 (±0.4)	9.9 (±0.2)	9.5 (±0.2)	9.7 (±0.3)
A 5%	11.2 (±0.4)	11.4 (±0.3)	11.5 (±0.6)	11.0 (±0.4)	10.2 (±0.6)	11.0 (±0.4)	10.5 (±0.3)	10.3 (±0.3)
Y 0.5%	8.6 (±0.5)	8.7 (±0.4)	8.8 (±0.4)	9.0 (±0.3)	8.0 (±0.9)	8.0 (±0.4)	7.3 (±0.3)	7.1 (±0.4)
Y 1%	9.4 (±0.3)	9.8 (±0.7)	9.5 (±0.4)	9.6 (±0.2)	8.0 (±0.5)	9.0 (±0.6)	8.6 (±0.1)	8.2 (±0.4)
Y 5%	10.4 (±0.5)	10.6 (±0.5)	10.7 (±0.3)	10.5 (±0.4)	9.0 (±0.9)	10.0 (±0.9)	9.5 (±0.2)	9.3 (±0.7)

Note: Figures in brackets represent standard deviation.

Table 2.2. pH of Prescot samples (Mean of three replicates).

	Soil pH				pH of leachate			
Sample	Initially	After one month	After two months	After three months	Initially	After one month	After two months	After three months
Unamended	**3.3 (±0.6)**	**4.0 (±0.7)**	**3.8 (±0.3)**	**3.5 (±0.2)**	**3.6 (±0.4)**	**4.6 (±0.3)**	**4.2 (±0.3)**	**4.3 (±0.3)**
P 0.5%	5.4 (±0.5)	5.8 (±0.7)	5.4 (±0.4)	5.5 (±0.3)	5.6 (±0.5)	6.6 (±0.3)	6.0 (±0.3)	6.5 (±0.2)
P 1%	6.1 (±0.2)	6.6 (±0.5)	6.5 (±0.3)	6.0 (±0.4)	6.9 (±0.2)	7.6 (±0.5)	7.0 (±0.2)	7.0 (±0.1)
P 5%	7.0 (±0.4)	8.0 (±0.8)	7.8 (±0.4)	7.2 (±0.5)	7.0 (±0.6)	8.4 (±0.4)	8.6 (±0.2)	8.0 (±0.4)
A 0.5%	5.6 (±0.5)	5.6 (±0.4)	5.5 (±0.4)	5.1 (±0.4)	4.8 (±0.5)	6.0 (±0.8)	6.4 (±0.3)	5.8 (±0.3)
A 1%	6.2 (±0.5)	6.4 (±0.3)	6.3 (±0.3)	6.0 (±0.3)	6.2 (±0.4)	7.6 (±0.3)	7.1 (±0.4)	7.0 (±0.4)
A 5%	7.2 (±0.5)	8.4 (±0.4)	8.2 (±0.3)	7.6 (±0.2)	7.2 (±0.3)	8.4 (±0.4)	7.7 (±0.3)	8.0 (±0.2)
Y 0.5%	3.9 (±0.4)	4.3 (±0.8)	4.2 (±0.4)	4.0 (±0.2)	4.1 (±0.5)	4.7 (±0.3)	5.4 (±0.4)	5.2 (±0.3)
Y 1%	4.3 (±0.4)	4.8 (±0.5)	4.7 (±0.3)	4.5 (±0.2)	4.4 (±0.5)	5.8 (±0.2)	6.0 (±0.4)	6.3 (±0.3)
Y 5%	5.2 (±0.3)	5.6 (±0.4)	5.3 (±0.4)	5.0 (±0.2)	5.0 (±0.3)	6.2 (±0.5)	6.5 (±0.3)	6.0 (±0.2)
+ 1% CaCO3	5.0 (±0.4)	5.6 (±0.2)	5.4 (±0.4)	5.1 (±0.2)	5.4 (±0.4)	6.3 (±0.4)	6.1 (±0.4)	6.0 (±0.3)

Note: Figures in brackets represent standard deviation.

Table 2.3. pH of Gateacre samples (Mean of three replicates).

	Soil pH				pH of leachate			
Sample	Initially	After one month	After two months	After three months	Initially	After one month	After two months	After three months
Unamended	**5.0 (±0.2)**	**5.6 (±0.5)**	**5.4 (±0.3)**	**5.3 (±0.6)**	**4.6 (±0.2)**	**7.4 (±0.4)**	**7.5 (±0.2)**	**7.0 (±0.2)**
P 0.5%	6.2 (±0.5)	6.8 (±0.5)	7.3 (±0.3)	6.8 (±0.2)	6.8 (±0.7)	7.6 (±0.5)	7.0 (±0.4)	7.2 (±0.3)
P 1%	6.9 (±0.4)	7.4 (±0.3)	8.0 (±0.4)	7.4 (±0.3)	6.8 (±0.5)	8.4 (±0.4)	7.8 (±0.2)	8.0 (±0.3)
P 5%	8.4 (±0.4)	8.8 (±0.2)	9.2 (±0.3)	8.7 (±0.3)	8.2 (±0.4)	9.0 (±0.2)	9.2 (±0.3)	8.3 (±0.3)
A 0.5%	6.4 (±0.4)	6.6 (±0.5)	7.1 (±0.2)	6.8 (±0.3)	5.6 (±0.5)	7.6 (±0.3)	7.4 (±0.4)	7.0 (±0.3)
A 1%	7.2 (±0.4)	7.4 (±0.4)	7.5 (±0.3)	7.5 (±0.2)	6.0 (±0.4)	7.8 (±0.3)	8.2 (±0.4)	8.0 (±0.3)
A 5%	8.2 (±0.4)	8.2 (±0.3)	8.0 (±0.6)	8.0 (±0.2)	7.8 (±0.3)	8.8 (±0.3)	8.5 (±0.2)	8.4 (±0.5)
Y 0.5%	4.8 (±0.3)	5.0 (±0.4)	5.7 (±0.3)	5.5 (±0.4)	5.2 (±0.4)	6.8 (±0.3)	6.9 (±0.4)	7.0 (±0.3)
Y 1%	5.0 (±0.5)	5.3 (±0.4)	5.0 (±0.3)	5.8 (±0.3)	5.4 (±0.4)	7.8 (±0.5)	8.0 (±0.2)	8.2 (±0.4)
Y 5%	6.0 (±0.3)	6.4 (±0.3)	6.0 (±0.2)	6.6 (±0.2)	6.6 (±0.5)	8.3 (±0.4)	7.5 (±0.3)	8.0 (±0.4)
+ 1% CaCO3	7.0 (±0.2)	7.4 (±0.2)	7.5 (±0.4)	7.2 (±0.2)	6.6 (±0.5)	8.0 (±0.4)	8.2 (±0.3)	8.4 (±0.4)

Note: Figures in brackets represent standard deviation.

Appendix 3

Table 3.1. Water extractable copper and lead for Trelogan soil solutions (Mean of three replicates).

	COPPER				LEAD			
Sample	Initially	After one month	After two months	After three months	Initially	After one month	After two months	After three months
Unamended (pH=7.4)	**0.60 (±0.2)**	**0.70 (±0.06)**	**0.75 (±0.04)**	**0.68 (±0.03)**	**100 (±13.3)**	**100 (±10)**	**100 (±13.3)**	**100 (±5.2)**
Unamended (pH=10.0)	**0.46 (±0.03)**	**0.50 (±0.02)**	**0.48 (±0.04)**	**0.52 (±0.06)**	**90 (±13)**	**90 (±7.2)**	**90 (±6.5)**	**90 (±8)**
P 0.5%	0.45 (±0.03)	0.38 (±0.03)	0.38 (±0.02)	0.36 (±0.04)	87 (±6)	78 (±7.2)	75 (±9)	80 (±5)
P 1%	0.48 (±0.03)	0.35 (±0.04)	0.33 (±0.03)	0.34 (±0.06)	85 (±5)	70 (±5)	68 (±7.2)	70 (±8.7)
P 5%	0.43 (±0.03)	0.30 (±0.02)	0.28 (±0.02)	0.31 (±0.03)	84 (±8)	55 (±7)	53 (±2.6)	54 (±5.6)
A 0.5%	0.44 (±0.02)	0.36 (±0.04)	0.35 (±0.03)	0.35 (±0.03)	86 (±6)	76 (±3.5)	75 (±4.4)	75 (±10.4)
A 1%	0.42 (±0.02)	0.34 (±0.05)	0.31 (±0.02)	0.33 (±0.04)	85 (±4.4)	64 (±5.3)	62 (±8.2)	60 (±5)
A 5%	0.43 (±0.03)	0.31 (±0.02)	0.30 (±0.04)	0.30 (±0.02)	86 (±5.3)	54 (±7.2)	56 (±6.6)	55 (±4.4)
Y 0.5%	0.46 (±0.01)	0.44 (±0.04)	0.42 (±0.03)	0.45 (±0.03)	90 (±8.5)	85 (±5)	82 (±8.2)	83 (±10)
Y 1%	0.45 (±0.03)	0.40 (±0.04)	0.40 (±0.03)	0.40 (±0.03)	87 (±7)	80 (±4.6)	80 (±4.6)	80 (±5)
Y 5%	0.42 (±0.03)	0.34 (±0.04)	0.31 (±0.03)	0.30 (±0.02)	85 (±11)	63 (±2.6)	62 (±3.5)	60 (±4)

Note: Figures in brackets represent standard deviation.

Table 3.2. Water extractable copper and lead for Prescot soil solutions (Mean of three replicates).

	COPPER				LEAD			
Sample	Initially	After one month	After two months	After three months	Initially	After one month	After two months	After three months
Unamended (pH=3.6)	**53 (±3.6)**	**51 (±3.6)**	**50 (±4.4)**	**52 (±2.6)**	**3.0 (±0.4)**	**2.8 (±0.2)**	**3.0 (±0.4)**	**3.0 (±0.4)**
Unamended (pH=6.5)	**45 (±3.6)**	**46 (±1)**	**45 (±4.4)**	**47 (±4.4)**	**2.5 (±0.5)**	**2.4 (±0.4)**	**2.4 (±1)**	**2.5 (±0.3)**
P 0.5%	44 (±3.6)	32 (±2.6)	32 (±1.7)	30 (±2.6)	2.4 (±0.8)	1.8 (±0.2)	2.0 (±0.4)	2.0 (±0.4)
P 1%	43 (±2.6)	25 (±2.6)	25 (±2.6)	24 (±1.7)	2.3 (±0.3)	1.6 (±0.2)	1.5 (±0.2)	1.5 (±0.4)
P 5%	43 (±1)	24 (±3.6)	23 (±2.6)	23 (±2.6)	2.0 (±0.7)	1.4 (±0.2)	1.4 (±0.1)	1.4 (±0.5)
A 0.5%	45 (±4.3)	35 (±3.6)	36 (±5.6)	36 (±1.7)	2.5 (±0.2)	2.0 (±0.2)	2.0 (±0.3)	2.0 (±0.4)
A 1%	44 (±1.7)	27 (±5.6)	26 (±2.2)	26 (±1.7)	2.4 (±0.4)	1.6 (±0.2)	1.6 (±0.1)	1.5 (±0.3)
A 5%	42 (±4.4)	25 (±1.7)	25 (±1.7)	25 (±2.6)	2.3 (±0.3)	1.3 (±0.3)	1.3 (±0.4)	1.4 (±0.2)
Y 0.5%	46 (±1.7)	44 (±4)	43 (±2.6)	43 (±2.6)	2.6 (±0.4)	2.2 (±0.2)	2.0 (±0.3)	2.0 (±0.3)
Y 1%	43 (±2.6)	41 (±1.7)	41 (±3.6)	40 (±2)	2.5 (±0.5)	2.0 (±0.3)	2.0 (±0.2)	2.0 (±0.3)
Y 5%	41 (±3.6)	26 (±1.7)	27 (±2.6)	25 (±2.6)	2.4 (±0.3)	1.5 (±0)	1.6 (±0.2)	1.5 (±0.2)
+ 1% CaCO3	43 (±8.2)	38 (±2.6)	41 (±2.6)	41 (±3.6)	2.6 (±0.5)	2.4 (±0.3)	2.5 (±0.4)	2.5 (±0.5)

Note: Figures in brackets represent standard deviation.

Table 3.3. Water extractable copper and lead for Gateacre soil solutions (Mean of three replicates).

	COPPER				LEAD			
Sample	Initially	After one month	After two months	After three months	Initially	After one month	After two months	After three months
Unamended (pH=4.6)	**1.7 (±0.3)**	**1.8 (±0.2)**	**1.7 (±0.1)**	**1.8 (±0.4)**	**5.0 (±1)**	**5.2 (±0.4)**	**5.0 (±0.3)**	**5.0 (±0.4)**
Unamended (pH=6.5)	**1.5 (±0.5)**	**1.4 (±0.3)**	**1.4 (±0.4)**	**1.5 (±0)**	**4.8 (±0.8)**	**5.0 (±0.4)**	**5.0 (±0.4)**	**4.8 (±0.7)**
P 0.5%	1.5 (±0.4)	1.0 (±0.3)	1.0 (±0.4)	1.0 (±0.2)	4.8 (±0.5)	4.2 (±0.7)	4.0 (±0.3)	4.0 (±0.3)
P 1%	1.4 (±0.1)	0.8 (±0.3)	0.8 (±0.2)	0.9 (±0.1)	4.6 (±0.7)	3.5 (±0.2)	3.6 (±0.4)	3.6 (±0.4)
P 5%	1.4 (±0.1)	0.7 (±0.1)	0.7 (±0)	0.7 (±0)	4.5 (±0.5)	3.0 (±0.3)	3.2 (±0.4)	3.3 (±0.7)
A 0.5%	1.4 (±0.3)	1.2 (±0.3)	1.2 (±0.3)	1.1 (±0.4)	4.8 (±0.4)	4.0 (±0.6)	4.0 (±0.2)	4.2 (±0.4)
A 1%	1.5 (±0.2)	0.8 (±0.1)	0.8 (±0.2)	0.8 (±0.2)	4.7 (±0.6)	3.4 (±0.7)	3.3 (±0.6)	3.5 (±0.4)
A 5%	1.4 (±0.4)	0.7 (±0.1)	0.7 (±0.1)	0.7 (±0.1)	4.6 (±0.4)	3.0 (±0.5)	3.0 (±0.6)	3.0 (±0.3)
Y 0.5%	1.6 (±0.2)	1.3 (±0.1)	1.3 (±0.2)	1.4 (±0)	5.0 (±2)	4.5 (±0.4)	4.7 (±0.2)	4.7 (±0.3)
Y 1%	1.6 (±0.3)	1.2 (±0.4)	1.2 (±0.3)	1.2 (±0.2)	4.8 (±0.7)	4.0 (±0.4)	4.2 (±0.5)	4.0 (±0.2)
Y 5%	1.5 (±0.3)	0.8 (±0.2)	0.8 (±0.1)	0.8 (±0.1)	4.4 (±1.2)	3.4 (±0.2)	3.5 (±0.4)	3.5 (±0.3)
+ 1% CaCO3	1.4 (±0.1)	1.0 (±0)	1.3 (±0.4)	1.3 (±0.1)	4.6 (±0.5)	4.5 (±0.2)	4.3 (±0.3)	4.4 (±0.5)

Note: Figures in brackets represent standard deviation.

Table 3.4. Water extractable cadmium and zinc for Trelogan soil solutions (Mean of three replicates).

	CADMIUM				ZINC			
Sample	Initially	After one month	After two months	After three months	Initially	After one month	After two months	After three months
Unamended (pH=7.4)	**8.5 (±0.9)**	**8.0 (±0.6)**	**8.2 (±0.7)**	**8.4 (±1)**	**70 (±4.6)**	**70 (±6)**	**70 (±6.3)**	**70 (±2.7)**
Unamended (pH=10.0)	**7.0 (±0.9)**	**6.8 (±0.7)**	**6.7 (±0.6)**	**7.0 (±1)**	**65 (±2.6)**	**60 (±5)**	**60 (±3.5)**	**60 (±6.6)**
P 0.5%	6.8 (±0.3)	5.5 (±0.5)	5.6 (±0.5)	5.4 (±0.5)	63 (±3)	45 (±5)	45 (±4.4)	45 (±3.6)
P 1%	6.6 (±0.4)	5.2 (±0.7)	5.0 (±0.5)	5.0 (±1)	61 (±1.7)	40 (±3.6)	40 (±4.4)	40 (±6.3)
P 5%	7.2 (±0.3)	4.2 (±0.7)	4.3 (±0.6)	4.4 (±0.5)	60 (±4.4)	35 (±3.6)	30 (±2.7)	30 (±1.8)
A 0.5%	7.1 (±0.4)	5.6 (±0.5)	5.5 (±1)	5.7 (±0.5)	62 (±5.3)	45 (±5.2)	50 (±1.8)	50 (±5.3)
A 1%	7.0 (±0.6)	5.4 (±0.4)	5.6 (±0.6)	5.1 (±0.6)	62 (±2)	40 (±3.5)	40 (±4.4)	40 (±3)
A 5%	6.7 (±0.6)	4.4 (±0.5)	4.2 (±0.7)	4.0 (±0.5)	61 (±5.3)	35 (±4.4)	30 (±3.5)	35 (±5.3)
Y 0.5%	7.5 (±0.5)	6.6 (±0.5)	6.7 (±0.3)	6.5 (±0.5)	64 (±5.3)	60 (±2.7)	60 (±2)	55 (±5)
Y 1%	7.3 (±1)	6.0 (±0.2)	6.2 (±1)	5.8 (±0.7)	60 (±5.3)	50 (±3.6)	50 (±1.8)	50 (±5.6)
Y 5%	7.0 (±0.3)	5.0 (±0.4)	5.1 (±0.6)	5.0 (±0.5)	60 (±8.6)	40 (±4.4)	40 (±3.6)	40 (±5.3)

Note: Figures in brackets represent standard deviation.

Table 3.5. Water extractable cadmium and zinc for Prescot soil solutions (Mean of three replicates).

	CADMIUM				ZINC			
Sample	Initially	After one month	After two months	After three months	Initially	After one month	After two months	After three months
Unamended (pH=3.6)	**0.8 (±0.2)**	**0.8 (±0.1)**	**0.8 (±0.1)**	**0.9 (±0.2)**	**30 (±6.6)**	**28 (±4.4)**	**26 (±6.6)**	**30 (±3)**
Unamended (pH=6.5)	**0.7 (±0.1)**	**0.7 (±0.1)**	**0.6 (±0.1)**	**0.7 (±0)**	**28 (±2.6)**	**26 (±1)**	**25 (±4.4)**	**26 (±5.6)**
P 0.5%	0.7 (±0)	0.4 (±0)	0.3 (±0)	0.3 (±0.2)	28 (±4.4)	14 (±3.6)	13 (±3.6)	13 (±5.2)
P 1%	0.6 (±0)	0.3 (±0.1)	0.3 (±0.1)	0.3 (±0.1)	27 (±2.6)	13 (±3.6)	11 (±1.7)	12 (±1.7)
P 5%	0.6 (±0.1)	0.3 (±0.1)	0.2 (±0)	0.3 (±0.1)	26 (±1)	10 (±1.8)	10 (±2.6)	10 (±5.3)
A 0.5%	0.7 (±0.1)	0.5 (±0)	0.5 (±0)	0.5 (±0.1)	28 (±2)	15 (±2.6)	14 (±2.6)	14 (±2.6)
A 1%	0.7 (±0.1)	0.4 (±0.1)	0.4 (±0.1)	0.4 (±0.1)	27 (±8.2)	13 (±1.3)	13 (±3.6)	12 (±1.7)
A 5%	0.7 (±0.2)	0.3 (±0.1)	0.3 (±0.1)	0.3 (±0.1)	27 (±4.4)	11 (±1.2)	12 (±3.5)	10 (±1)
Y 0.5%	0.7 (±0)	0.6 (±0.1)	0.6 (±0.1)	0.6 (±0.2)	28 (±2.6)	20 (±6.6)	20 (±3.5)	19 (±3.6)
Y 1%	0.7 (±0.1)	0.5 (±0.1)	0.6 (±0)	0.6 (±0.2)	28 (±8.5)	18 (±1.7)	19 (±1.7)	17 (±2.6)
Y 5%	0.7 (±0.1)	0.4 (±0.1)	0.4 (±0)	0.4 (±0.1)	26 (±5.6)	13 (±2.6)	13 (±1.3)	11 (±2.6)
+ 1% CaCO3	0.6 (±0.2)	0.5 (±0.1)	0.5 (±0.1)	0.5 (±0.1)	26 (±1.7)	22 (±5.8)	24 (±4.6)	23 (±5.2)

Note: Figures in brackets represent standard deviation.

Table 3.6. Water extractable cadmium and zinc for Gateacre soil solutions (Mean of three replicates).

	CADMIUM				ZINC			
Sample	Initially	After one month	After two months	After three months	Initially	After one month	After two months	After three months
Unamended (pH=4.6)	**1.7 (±0.3)**	**1.8 (±0.3)**	**1.7 (±0.3)**	**1.7 (±0.3)**	**40 (±2.6)**	**40 (±3.5)**	**38 (±6.2)**	**40 (±4.6)**
Unamended (pH=6.5)	**1.3 (±0.3)**	**1.2 (±0.2)**	**1.2 (±0.2)**	**1.3 (±0.3)**	**38 (±10)**	**37 (±4.4)**	**35 (±4.4)**	**36 (±5.2)**
P 0.5%	1.3 (±0)	0.9 (±0.1)	0.8 (±0.2)	0.8 (±0.2)	37 (±4.4)	24 (±1.7)	25 (±1.7)	24 (±4.4)
P 1%	1.2 (±0.3)	0.7 (±0.1)	0.7 (±0.1)	0.7 (±0.1)	36 (±4)	22 (±2.6)	20 (±2.6)	22 (±3.6)
P 5%	1.2 (±0)	0.6 (±0.2)	0.6 (±0.1)	0.6 (±0.1)	36 (±5.3)	17 (±3)	18 (±1.7)	17 (±1.7)
A 0.5%	1.3 (±0.3)	0.8 (±0)	0.8 (±0)	0.8 (±0)	36 (±3.5)	25 (±2.6)	23 (±2.6)	22 (±5.3)
A 1%	1.3 (±0.3)	0.6 (±0.1)	0.6 (±0.1)	0.7 (±0)	38 (±2)	20 (±2)	18 (±2)	19 (±1)
A 5%	1.3 (±0)	0.5 (±0)	0.5 (±0.1)	0.5 (±0.1)	35 (±2.6)	17 (±2.6)	16 (±1.7)	15 (±1.7)
Y 0.5%	1.3 (±0.1)	1.0 (±0.1)	0.9 (±0.1)	0.9 (±0.2)	40 (±1.7)	30 (±1.7)	30 (±4.4)	30 (±2.6)
Y 1%	1.3 (±0.5)	0.9 (±0)	0.8 (±0.1)	0.8 (±0.1)	38 (±7)	25 (±1.7)	23 (±2.6)	25 (±2.6)
Y 5%	1.3 (±0.3)	0.7 (±0.1)	0.7 (±0.3)	0.7 (±0.1)	38 (±2)	22 (±2)	20 (±1.7)	21 (±3.6)
+ 1% CaCO3	1.3 (±0.2)	1.0 (±0.4)	1.0 (±0.3)	1.0 (±0.3)	36 (±3.6)	29 (±2.6)	34 (±5)	30 (±2.6)

Note: Figures in brackets represent standard deviation.

Appendix 4

Table 4.1. Treatment effects on yield, tissue metal concentrations, and metal uptake for ryegrass foliage grown in zeolite-amended soils.

	PRESCOT									GATEACRE								
		Tissue concentrations				Uptake					Tissue concentrations				Uptake			
Treatment	Yield	Cu	Cd	Zn	Pb	Cu	Cd	Zn	Pb	Yield	Cu	Cd	Zn	Pb	Cu	Cd	Zn	Pb
	g/pot	mg/kg				mg/pot				g/pot	mg/kg				mg/pot			
Compost	12.4	61.5	0.03	70	56	0.76	0.0004	0.87	0.7	12.4	61.5	0.03	70	56	0.76	0.0004	0.87	0.7
Unamended soil	1.12	180	2.5	130	100	0.2	0.003	0.15	0.11	10.7	20	6	100	120	0.21	0.064	1.1	1.3
Zeo P 0.5%	3.9	90	1.5	35	70	0.35	0.006	0.14	0.27	16.3	10	4	70	75	0.16	0.065	1.2	1.2
Zeo P 1%	6.42	140	1.5	45	65	0.9	0.01	0.29	0.42	14.85	12	3	65	80	0.18	0.044	0.96	1.2
Zeo P 5%	0.77	100	2	50	85	0.08	0.002	0.04	0.07	0.64	15	4.5	80	90	0.01	0.003	0.05	0.06
Zeo 4A 0.5%	5.65	100	1.7	35	65	0.57	0.01	0.2	0.37	18.1	10	3	55	80	0.18	0.054	1	1.45
Zeo 4A 1%	6.65	65	1.4	40	60	0.43	0.01	0.27	0.4	17.5	10	2.5	45	70	0.18	0.044	0.79	1.22
Zeo 4A 5%	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	6.5	12	3.5	75	75	0.08	0.023	0.49	0.49
Zeo Y 0.5%	3.23	110	2	65	90	0.35	0.006	0.21	0.29	13.53	18	5.5	75	75	0.24	0.074	1	1
Zeo Y 1%	6.4	90	2	60	85	0.58	0.013	0.38	0.54	16.9	12	5	80	80	0.2	0.084	1.35	1.35
Zeo Y 5%	5.6	120	1.7	55	75	0.67	0.01	0.31	0.42	18.15	15	4.5	60	65	0.27	0.081	1.1	1.18

Note: N.G. = No plant growth.

Table 4.2. Treatment effects on yield, tissue metal concentrations, and metal uptake for sunflower grown in Prescot soil.

	ROOTS									FOLIAGE								
		Tissue concentrations				Uptake					Tissue concentrations				Uptake			
Treatment	Yield	Cu	Cd	Zn	Pb	Cu	Cd	Zn	Pb	Yield	Cu	Cd	Zn	Pb	Cu	Cd	Zn	Pb
	g/pot	mg/kg				mg/pot				g/pot	mg/kg				mg/pot			
Compost	2.61	70	0.08	147.5	83	0.2	0.0002	0.38	0.22	8.92	98	0.05	100	57.4	0.87	0.00045	0.9	0.51
Unamended soil	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.
Zeo P 0.5%	0.88	600	4.5	300	110	0.53	0.004	0.264	0.1	2.28	180	2.5	150	40	0.41	0.006	0.34	0.1
Zeo P 1%	1.41	550	4	300	65	0.78	0.0056	0.423	0.1	2.62	165	1.5	200	25	0.43	0.004	0.52	0.065
Zeo P 5%	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.
Zeo 4A 0.5%	1.72	625	3	300	130	1.1	0.0052	0.52	0.22	6	250	1.8	170	55	1.5	0.011	1	0.33
Zeo 4A 1%	0.61	600	4	250	120	0.6	0.004	0.25	0.12	2.05	175	1.5	200	75	0.36	0.003	0.41	0.15
Zeo 4A 5%	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.
Zeo Y 0.5%	1.3	650	3	350	170	0.9	0.0011	0.455	0.22	3.4	130	1.7	130	30	0.44	0.006	0.44	0.1
Zeo Y 1%	0.71	400	4	300	160	0.4	0.0028	0.21	0.11	2	120	1.8	250	60	0.24	0.004	0.5	0.12
Zeo Y 5%	0.32	300	2.5	300	150	0.3	0.0025	0.3	0.15	1.97	155	1.4	250	75	0.3	0.003	0.49	0.15

Note: N.G.= No plant growth.

Table 4.3. Treatment effects on yield, tissue metal concentrations, and metal uptake for sunflower grown in Gateacre soil.

	ROOTS									FOLIAGE								
		Tissue concentrations				Uptake					Tissue concentrations				Uptake			
Treatment	Yield	Cu	Cd	Zn	Pb	Cu	Cd	Zn	Pb	Yield	Cu	Cd	Zn	Pb	Cu	Cd	Zn	Pb
	g/pot	mg/kg				mg/pot				g/pot	mg/kg				mg/pot			
Compost	2.61	70	0.08	147.5	83	0.2	0.0002	0.38	0.22	8.92	98	0.05	100	57.4	0.87	0.00045	0.9	0.51
Unamended soil	2.05	70	15	230	150	0.14	0.031	0.47	0.31	8.76	30	9	125	75	0.26	0.079	1.1	0.66
Zeo P 0.5%	1.7	45	10	100	100	0.08	0.017	0.17	0.17	6.4	18	4	60	45	0.12	0.026	0.38	0.29
Zeo P 1%	2.65	50	9	90	130	0.13	0.024	0.24	0.34	9.73	20	3	80	50	0.2	0.029	0.78	0.49
Zeo P 5%	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	1.06	22	4.4	65	65	0.023	0.047	0.69	0.07
Zeo 4A 0.5%	3.14	30	9.7	100	100	0.1	0.03	0.31	0.31	11	18	3.3	60	65	0.2	0.036	0.66	0.71
Zeo 4A 1%	0.33	50	10	110	80	0.05	0.01	0.11	0.08	2.41	25	2.7	70	50	0.06	0.0065	0.17	0.12
Zeo 4A 5%	0.54	40	10	80	150	0.04	0.01	0.08	0.15	2	18.5	3.8	60	55	0.037	0.0076	0.12	0.11
Zeo Y 0.5%	1.22	55	9.5	130	130	0.07	0.01	0.16	0.16	6	25	3.3	80	50	0.15	0.002	0.48	0.3
Zeo Y 1%	1	60	9.2	130	120	0.06	0.01	0.13	0.12	6.66	20	4	65	70	0.13	0.027	0.43	0.47
Zeo Y 5%	2.47	40	8.5	90	100	0.1	0.021	0.22	0.25	7.66	18.5	2.8	75	50	0.14	0.021	0.57	0.38

Note: N.G.= No plant growth.

Table 4.4. Treatment effects on yield, tissue metal concentrations, and metal uptake for maize grown in Prescot soil.

	ROOTS									FOLIAGE								
		Tissue concentrations				Uptake					Tissue concentrations				Uptake			
Treatment	Yield	Cu	Cd	Zn	Pb	Cu	Cd	Zn	Pb	Yield	Cu	Cd	Zn	Pb	Cu	Cd	Zn	Pb
	g/pot	mg/kg				mg/pot				g/pot	mg/kg				mg/pot			
Compost	3.01	70	0.07	100	70	0.21	0.0002	0.3	0.21	12.32	28	0.04	90	45	0.35	0.00049	1.1	0.55
Unamended soil	0.17	1000	3.5	300	160	0.1	0.0035	0.3	0.16	0.34	220	2	100	55	0.22	0.002	0.1	0.055
Zeo P 0.5%	1.87	700	2.5	150	90	1.31	0.0047	0.28	0.17	5.48	180	1	60	30	1	0.0055	0.33	0.164
Zeo P 1%	2.68	850	1.5	200	90	2.28	0.004	0.54	0.24	6.34	200	1.5	75	35	1.27	0.0095	0.48	0.222
Zeo P 5%	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.
Zeo 4A 0.5%	2.72	650	2.5	200	100	1.77	0.007	0.54	0.27	5.95	200	1	65	45	1.2	0.006	0.39	0.27
Zeo 4A 1%	4.03	800	2	150	80	3.22	0.008	0.6	0.32	8	160	1	60	30	1.28	0.008	0.48	0.24
Zeo 4A 5%	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.
Zeo Y 0.5%	0.11	750	2.5	250	110	0.75	0.0025	0.25	0.11	0.28	110	1.5	70	35	0.11	0.0015	0.07	0.035
Zeo Y 1%	0.4	650	2	250	100	0.65	0.002	0.25	0.1	1.9	185	1.5	60	60	0.35	0.0028	0.11	0.114
Zeo Y 5%	0.74	550	1.5	200	90	0.55	0.001	0.15	0.07	4.25	115	1.5	55	55	0.49	0.0064	0.23	0.23

Note: N.G.= No plant growth.

Table 4. 5. Treatment effects on yield, tissue metal concentrations, and metal uptake for maize grown in Gateacre soil.

	ROOTS									FOLIAGE								
		Tissue concentrations				Uptake					Tissue concentrations				Uptake			
Treatment	Yield	Cu	Cd	Zn	Pb	Cu	Cd	Zn	Pb	Yield	Cu	Cd	Zn	Pb	Cu	Cd	Zn	Pb
	g/pot	mg/kg				mg/pot				g/pot	mg/kg				mg/pot			
Compost	3.01	70	0.07	100	70	0.21	0.0002	0.3	0.21	12.32	28	0.04	90	45	0.35	0.00049	1.1	0.06
Unamended soil	4.37	90	18	300	175	0.40	0.079	1.31	0.76	13.23	30	8	130	70	0.4	0.11	1.72	0.93
Zeo P 0.5%	2.7	40	10	180	95	0.11	0.027	0.48	0.26	8.44	23	6	90	35	0.2	0.0051	0.76	0.3
Zeo P 1%	0.39	40	12	170	90	0.04	0.012	0.17	0.1	1.81	12.5	6.5	90	50	0.022	0.02	0.16	0.91
Zeo P 5%	0.35	80	13	120	100	0.08	0.013	0.12	0.1	0.32	12.5	7	60	50	0.013	0.007	0.06	0.05
Zeo 4A 0.5%	4.5	40	12	200	100	0.18	0.054	0.9	0.45	10.27	19	6.2	60	50	0.2	0.0064	0.62	0.51
Zeo 4A 1%	4.05	40	11	170	110	0.16	0.045	0.7	0.44	8.18	19	6.3	50	65	0.155	0.051	0.41	0.53
Zeo 4A 5%	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	0.27	12.5	6.2	100	70	0.013	0.0062	0.1	0.07
Zeo Y 0.5%	1.68	35	11	170	160	0.06	0.018	0.28	0.27	5.66	27	6	90	70	0.153	0.034	0.51	0.4
Zeo Y 1%	6.91	40	10	180	150	0.27	0.07	1.24	1	9.72	23	3	85	65	0.223	0.029	0.83	0.63
Zeo Y 5%	0.53	40	9	150	130	0.04	0.01	0.15	0.13	4	25	3	80	60	0.1	0.012	0.32	0.24

Note: N.G.= No plant growth.

Table 4.6. Treatment effects on yield, tissue metal concentrations, and metal uptake for willows grown in Prescot soil.

	SHOOTS									LEAVES								
		Tissue concentrations				Uptake					Tissue concentrations				Uptake			
Treatment	Yield	Cu	Cd	Zn	Pb	Cu	Cd	Zn	Pb	Yield	Cu	Cd	Zn	Pb	Cu	Cd	Zn	Pb
	g/pot	mg/kg				mg/pot				g/pot	mg/kg				mg/pot			
Compost	1.37	26.5	0.07	36	13	0.04	0.0009	0.05	0.02	3.16	16	0.12	42	12	0.05	0.00038	0.13	0.038
Unamended soil	1.24	140	18	175	60	0.174	0.022	0.22	0.07	2.1	100	20	200	150	0.21	0.042	0.42	0.32
Zeo P 0.5%	1.34	80	9	160	50	0.11	0.012	0.21	0.07	3	55	14	160	135	0.17	0.042	0.48	0.41
Zeo P 1%	1.38	50	15	130	45	0.07	0.021	0.18	0.06	3.1	55	12	180	130	0.17	0.037	0.56	0.4
Zeo P 5%	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.
Zeo 4A 0.5%	0.86	125	12	135	48	0.11	0.01	0.12	0.04	2.74	65	16	180	145	0.18	0.044	0.5	0.4
Zeo 4A 1%	1	135	14	155	45	0.14	0.014	0.16	0.05	2.58	60	17	170	125	0.16	0.044	0.44	0.32
Zeo 4A 5%	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.
Zeo Y 0.5%	0.45	135	13	150	60	0.14	0.013	0.15	0.06	1.16	85	14	190	125	0.1	0.016	0.22	0.15
Zeo Y 1%	1.09	100	17	155	55	0.11	0.018	0.17	0.06	2.5	75	12	150	150	0.19	0.03	0.38	0.38
Zeo Y 5%	0.38	70	15	165	40	0.07	0.015	0.17	0.04	0.63	80	13	165	100	0.05	0.008	0.1	0.06

Note: N.G.= No plant growth.

Table 4.7. Treatment effects on yield, tissue metal concentrations, and metal uptake for willows grown in Gateacre soil.

	SHOOTS									LEAVES								
		Tissue concentrations				Uptake					Tissue concentrations				Uptake			
Treatment	Yield	Cu	Cd	Zn	Pb	Cu	Cd	Zn	Pb	Yield	Cu	Cd	Zn	Pb	Cu	Cd	Zn	Pb
	g/pot	mg/kg				mg/pot				g/pot	mg/kg				mg/pot			
Compost	1.37	26.5	0.67	36.3	13	0.04	0.0009	0.05	0.02	3.16	16	0.12	40	12	0.05	0.0004	0.14	0.04
Unamended soil	1.14	70	6.5	200	45	0.08	0.007	0.23	0.05	2.47	65	22	100	180	0.16	0.054	0.25	0.044
Zeo P 0.5%	1.21	45	5	110	35	0.054	0.006	0.13	0.04	3.36	35	22	55	130	0.12	0.074	0.19	0.44
Zeo P 1%	1.71	35	6	120	40	0.06	0.01	0.20	0.07	2.8	50	18	70	170	0.14	0.05	0.2	0.48
Zeo P 5%	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.
Zeo 4A 0.5%	1.28	40	4.5	100	40	0.05	0.006	0.13	0.05	3.7	45	12	55	150	1.17	0.044	0.2	0.56
Zeo 4A 1%	1.62	55	4.5	120	35	0.09	0.007	0.2	0.06	2.61	40	18	50	140	0.1	0.05	0.13	0.37
Zeo 4A 5%	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.
Zeo Y 0.5%	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.
Zeo Y 1%	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.
Zeo Y 5%	1.6	60	5	155	40	0.1	0.008	0.25	0.06	3.9	35	14	60	145	0.14	0.055	0.23	0.57

Note: N.G.= No plant growth.

Appendix 5

Table 5.1. ANOVA (One-way) and Tukey's Test.

Code	Zeolite	Concentration (%)
1	-	-
2	P	0.5
3	P	1
4	P	5
5	4A	0.5
6	4A	1
7	4A	5
8	Y	0.5
9	Y	1
10	Y	5

Treatment	F	P
Cu Rg F P	16.46	0.000
Cu Rg F G	3.26	0.040
Cd Rg F P	1.54	0.277
Cd Rg F G	7.44	0.002
Zn Rg F P	9.76	0.002
Zn Rg F G	10.16	0.001
Pb Rg F P	4.48	0.024
Pb Rg F G	6.33	0.004
Cu Sf R P	6.66	0.044
Cu Sf R G	5.26	0.021
Cd Sf R P	1.90	0.279
Cd Sf R G	7.89	0.007
Zn Sf R P	2.06	0.253
Zn Sf R G	8.86	0.005
Pb Sf R P	10.20	0.021
Pb Sf R G	9.33	0.004
Cu Sf F P	13.46	0.002
Cu Sf F G	17.80	0.000
Cd Sf F P	1.06	0.463
Cd Sf F G	43.46	0.000
Zn Sf F P	10.25	0.004
Zn Sf F G	10.99	0.000
Pb Sf F P	17.78	0.001
Pb Sf F G	3.48	0.033

Table 5.1. continued

Cu M R P	37.19	0.001
Cu M R G	10.59	0.005
Cd M R P	2.98	0.124
Cd M R G	2.72	0.119
Zn M R P	3.67	0.086
Zn M R G	10.20	0.005
Pb M R P	5.62	0.038
Pb M R G	4.01	0.054
Cu M F P	5.99	0.015
Cu M F G	7.53	0.005
Cd M F P	1.14	0.433
Cd M F G	2.59	0.097
Zn M F P	16.52	0.001
Zn M F G	7.25	0.005
Pb M F P	24.18	0.000
Pb M F G	5.95	0.010
Cu W S P	2.73	0.105
Cu W S G	4.74	0.021
Cd W S P	1.95	0.199
Cd W S G	1.62	0.250
Zn W S P	6.71	0.011
Zn W S G	14.82	0.000
Pb W S P	2.02	0.186
Pb W S G	1.09	0.426
Cu W L P	5.98	0.002
Cu W L G	8.61	0.001
Cd W L P	11.71	0.181
Cd W L G	3.70	0.029
Zn W L P	4.88	0.005
Zn W L G	5.34	0.000
Pb W L P	2.19	0.100
Pb W L G	2.43	0.097

Note: Rg=ryegrass; Sf=sunflower; M=maize; W=willows; P=Prescot; G=Gateacre; R=roots; F=foliage; S=shoots; L=leaves.